GENERAL INFORMATION

MAY 18 1957

JOURNAL OF DAIRY SCIENCE

APRIL, 1957

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Literature Abstracts

ANNUAL MEETING
June 26-29, '57
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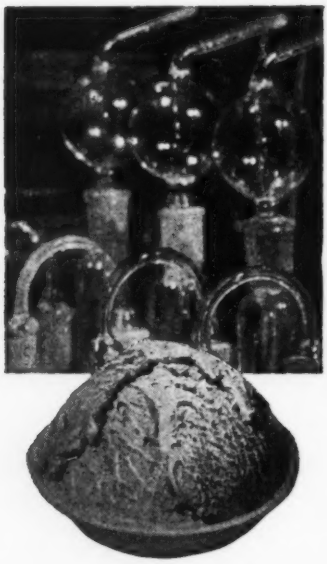

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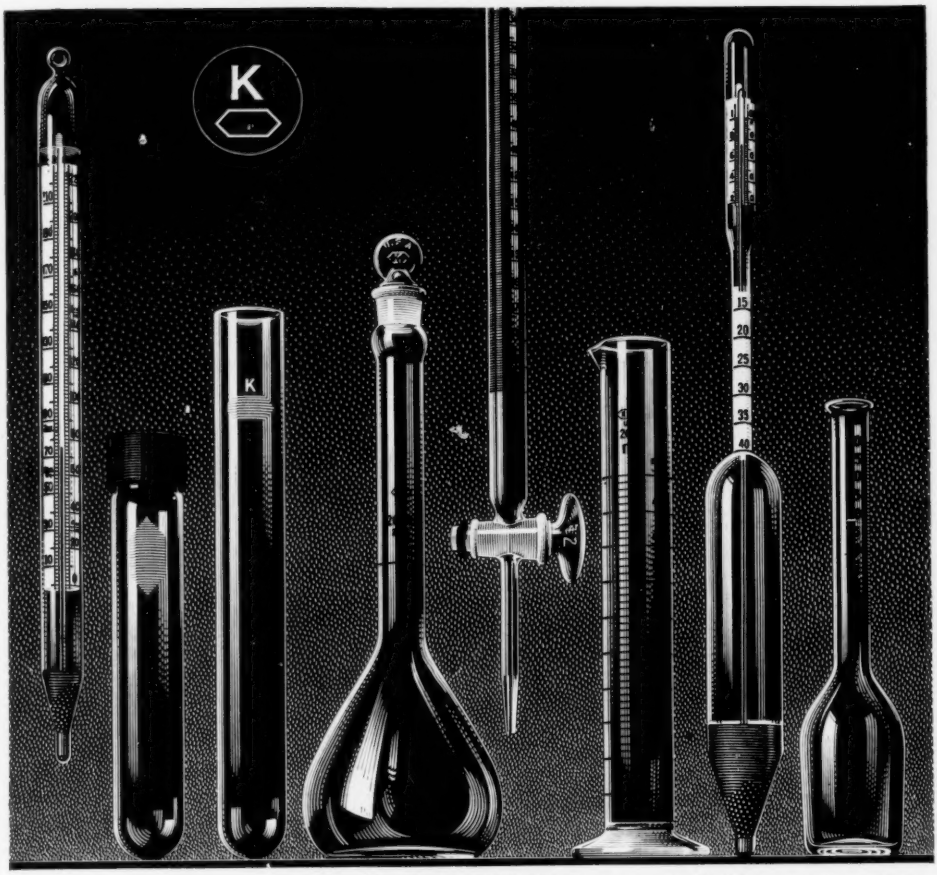

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Manuscripts for publication in the Journal of Dairy Science should be submitted to the editor, E. O. Herreid, at the address above. See January, 1954, issue, page 103, for information concerning the preparation of manuscripts, or send for reprint, "Information for Authors."

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Advertising plates, copy, insertion orders, contracts, and requests for information relating to advertising in the Journal should be addressed to The Garrard Press, 119-123 West Park Ave., Champaign, Ill.

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Printed by The Garrard Press, 119-123 West Park Avenue, Champaign, Illinois. Entered as second-class matter at the post office at Champaign, Illinois, under the act of March 3, 1879.

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JOURNAL OF DAIRY SCIENCE

VOLUME XL | April, 1957 | NUMBER 4

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PEOPLE *and* EVENTS
in the Dairy Science World

Federation Protests Butter Oil Proposal

A U. S. Tariff Commission recommendation to the President on March 11 that butter oil imports be set at 1,800,000 pounds per year was being protested to the White House this month by the National Milk Producers Federation. The annual quota would become effective July 1, with an interim quota from April 1 to June 30 of 450,000 pounds. The Federation stated:

> Butter oil imports at these figures would necessitate the government purchase of an additional 2,250,000 pounds of butter per year—a needless burden on the budget and one which would create further criticism of the price-support program. The Department of Agriculture, it was pointed out, had recommended the complete restriction of butter oil imports.

The Federation became concerned with the butter oil situation last year when the product—classified for customs purposes as a butter substitute—began to be imported in substantial quantities in circumvention of the intent of Section 22 of the Agricultural Adjustment Act. The Federation requested the Secretary of Agriculture to initiate action for import controls; the Secretary requested the President to ask for an investigation, and the Tariff Commission held hearings. The Commission's recommendation is subject to approval, disapproval, or modification by the President.

Cator Retires from Dean Milk Company; Corbett and Esmond Named Vice-Presidents

Dean Milk Company, Franklin Park, Ill., has announced the retirement of CLINTON C. CATOR, vice-president, effective March 1, 1957, and the election of WILLARD J. CORBETT and RAY ESMOND to the positions of vice-president.

Mr. Cator served with Dean Milk Company for 30 years. In 1939 he was elected to the Board of Directors and became a vice-president in 1940. He remains a member of the Board and continues to serve Dean Milk Company in a consulting capacity.

Dr. Corbett has been with Dean Milk Company since 1943 and is a member of the Board of Directors. Mr. Esmond has been with Dean Milk Company since 1929 and for the past several years has held the position of Fluid Milk Production Manager.

Ohio State News

"Personnel" was the topic discussed at the March meetings of the four Ohio Dairy Technology Societies. The speakers were G. E. Foxx, manager of industrial relations, Westinghouse Corporation, Lima, who spoke before the Maumee Valley Society; ROBERT M. MYERS, manager of industrial relations, Westinghouse Corporation, Cleveland, who spoke before the Cleveland Society, and E. ARTER, personnel manager, Nationwide Insurance Company, Columbus, who spoke before the Central Ohio and Cincinnati Societies.

News from Borden's

Record sales, earnings, and tonnage for the Borden Company in 1957 have been forecast by THEODORE G. MONTAGUE, chairman, and HAROLD W. COMFORT, president. In the annual report released recently, they based their expectation for the increase on an expanding economy, as well as the stepped-up activities within the company as part of its Centennial program.

Sales in 1956, the report noted, were the highest in the company's history. They totaled $876,987,184, an increase of 8.3 per cent over the previous high of $810,126,624, set in 1955. The volume of products handled last year was also the highest on record.

Net income in 1956 was $23,602,746. This was nine per cent more than the $21,653,536 in 1955. Earnings were $5.01 per share on the 4,707,000 shares outstanding Dec. 31, against $4.61 per share on 4,692,000 shares outstanding a year earlier. The rate of profit was 2.7 cents per dollar of sales, compared with a 20-year average of 2.9 cents.

The increase in sales did not result in a proportionately larger increase in profits, because of declines in selling prices of some principal products without compensating decreases in costs; increased promotional expenditures because of wider coverage; greater competition and higher costs, and a greater outlay for research. The company completed its 58th year of uninterrupted dividend payments in 1956. Dividends totaled $2.80 per share, the same as in 1955.

During 1956 the company began the largest research program in its history, the report said. Construction got under way on a $1,000,000 Research and Development Center at Syracuse, N. Y., where all phases of product development and improvement will be carried out. Several

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technical service laboratories for chemical products were established. An Executive Research Committee, with Mr. Comfort as chairman, was organized "to encourage the research activities of the entire Company."

During 1956 the company expanded its physical facilities by about $23,800,000. The program was financed by $13,600,000 in depreciation accruals, and $4,400,000 from working capital. Facilities with a value of about $5,800,000, principally motor vehicles and refrigerated cabinets, were obtained under leasing arrangements. In 1957, physical facilities will be expanded and modernized to the extent of about $28,300,000.

A new ice cream plant at Columbus, O., and Borden's first polyvinyl chloride resin plant, Leominster, Mass., went into operation last year. During 1957, the company will complete new fluid milk plants at Abilene, Tex., and Jackson, Miss.; and construction will get under way on milk, ice cream, or combination milk-ice cream plants at Beaumont and Amarillo, Tex., Baton Rouge, La., Orlando, Fla., Syracuse, N. Y., and Pittsburgh, Pa. Formaldehyde plants at Kent, Wash., and Fayetteville, N. C., and a synthetic resins plant in the Philippines, also will open in 1957.

Completed Theses

M. S. Degree

HARLEY D. HATHAWAY—The effect of concentration uptake at calving time on physiological activities with special emphasis on ketosis. The Ohio State Univ., Columbus.

D. C. VELL—Milk fat control in Ohio dairy plants. The Ohio State Univ., Columbus.

Ph.D. Degree

R. A. REISFELD—A study of the calcium-binding properties of whole casein, alpha-casein, and beta-casein. The Ohio State Univ., Columbus.

Penn State News

JOHN O. ALMQUIST, head of the Dairy Breeding Research Center, spent most of last summer in Europe, giving papers at the International Congress on Animal Reproduction in England and visiting universities and artificial breeding associations in Great Britain, Norway, Sweden, The Netherlands, France, Switzerland, and Italy.

HOWARD W. THOELE, who has served as a dairy specialist in extension work for the past two years, has transferred to the resident instruction staffi in Dairy Science. He will teach and do research in breeding, selection, and judging of dairy cattle. Dr. Thoele received all of his scientific training at the Univ. of Minnesota, St. Paul.

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STUART PATTON, a staff member in dairy science research, was recently honored by the American Chemical Society with the Borden Award in Dairy Chemistry for 1957, at the Miami, Fla., meetings.

HOWARD W. HAWK, '51 dairy production graduate, completed his Ph.D. program at the Univ. of Wisconsin, Madison, and recently has accepted a position as physiologist at the Dairy Husbandry Research Branch, USDA, Beltsville, Md.

PAUL S. WILLIAMS, a long-time member of the dairy production staff, is presently enjoying a well-earned sabbatical leave (first one in 36 years). Paul expects to tour the country by car and trailer, to visit some 25 colleges and experiment stations.

E. A. DAY is now at the Univ. of Maryland, College Park, his Alma Mater, as a member of the Dairy Dept.'s staff in dairy manufacturing. He received his Ph.D. from Penn State in 1956.

Dairy Price-Support Purchases

Dairy price-support purchases in February amounted to 10,061,996 pounds of butter, or approximately 3.2 million pounds less than in the same month last year. The CCC sold an additional 197,652 pounds of butter for commercial export, making the total in this classification since June, 1954, 30,823,548 pounds. USDA also reported sales of 100,874 pounds of Government butter for restrictive use of extending cocoa butter—sales for this use now total 6,501,220 pounds.

News from Louisiana State

Dairy quality control was the theme of the annual Dairy Technology Short Course at Louisiana State Univ., Baton Rouge, March 11-12. Quality control men from dairy plants in all parts of the state, together with representatives from the State Board of Health, attended the course. A. J. GELPI, JR. presided as general chairman.

In-place cleaning of dairy plant equipment, because of its growing popularity, took first place in point of interest among the subjects discussed and demonstrated. Practical tests—chemical, physical and bacterial—were given a lot of attention during the session. On the program, besides faculty members, were two guest speakers, J. W. GOODART, The Borden Company, and C. A. ABELE, Diversey Corporation. On the second day, a luncheon was sponsored by local dairy plants for the dairymen, faculty, and guests.

That this was the best short course ever offered by the Dairy Dept., mainly because the whole program centered on the one main topic, was the unanimous opinion of those who attended.

Recent Deaths

A. LELAND BEAM, for 40 years a member of the dairy production staff at Pennsylvania State Univ., University Park, died in March at his home, after a long illness. In addition to long service with the dairy production staff, Mr. Beam had been Director of Short Courses for the College of Agriculture.

ROBERT E. JONES, 68, publisher of the *Western Dairy Foods Review*, died suddenly of a heart attack February 9 at Berkeley, California.

His early training was in the newspaper business, and during his career he was editor, publisher, author, industry sales manager, association manager, and a specialist in public relations. In 1956, he went to Spain with a dairy exhibit installed by the United States Office of Information at the Barcelona Trade Fair.

Mr. Jones is survived by his widow, Mrs. ADA B. JONES, Berkeley, California, two sons, and a daughter.

MSU Will Conduct Feed Symposium

A National Symposium on *"The Nutritional and Economic Aspects of Feed Utilization by Dairy Cows"* will be held at Michigan State Univ., East Lansing, May 23, 24, and 25, 1957. The Depts. of Dairy Husbandry and Agricultural Economics at Michigan State Univ. will sponsor the symposium.

Purpose of the meeting is to provide better understanding between the dairymen and the economists, as well as other people who are working in this area. The ultimate aim of the proposed symposium is to stimulate an exchange of ideas, with the hope that more cooperative research throughout the country will be forthcoming.

The symposium is divided into five major areas: (*1*) Framework for the Symposium; (*2*) Adequacy of Dairy Feeding Input-Output Research Data; (*3*) Nutritional Consideration; (*4*) Designing Feeding Experiments, and (*5*) Application of Dairy Feeding Efficiency Research Data.

Twenty-five of the leading researchers from throughout the United States will participate.

Land-Grant Colleges Lack Sufficient Number of Ag Grads

Although there are fewer farmers every year, jobs relating to agriculture are multiplying, and land-grant colleges will fall short this year in number of graduates sufficient to fill the current demand.

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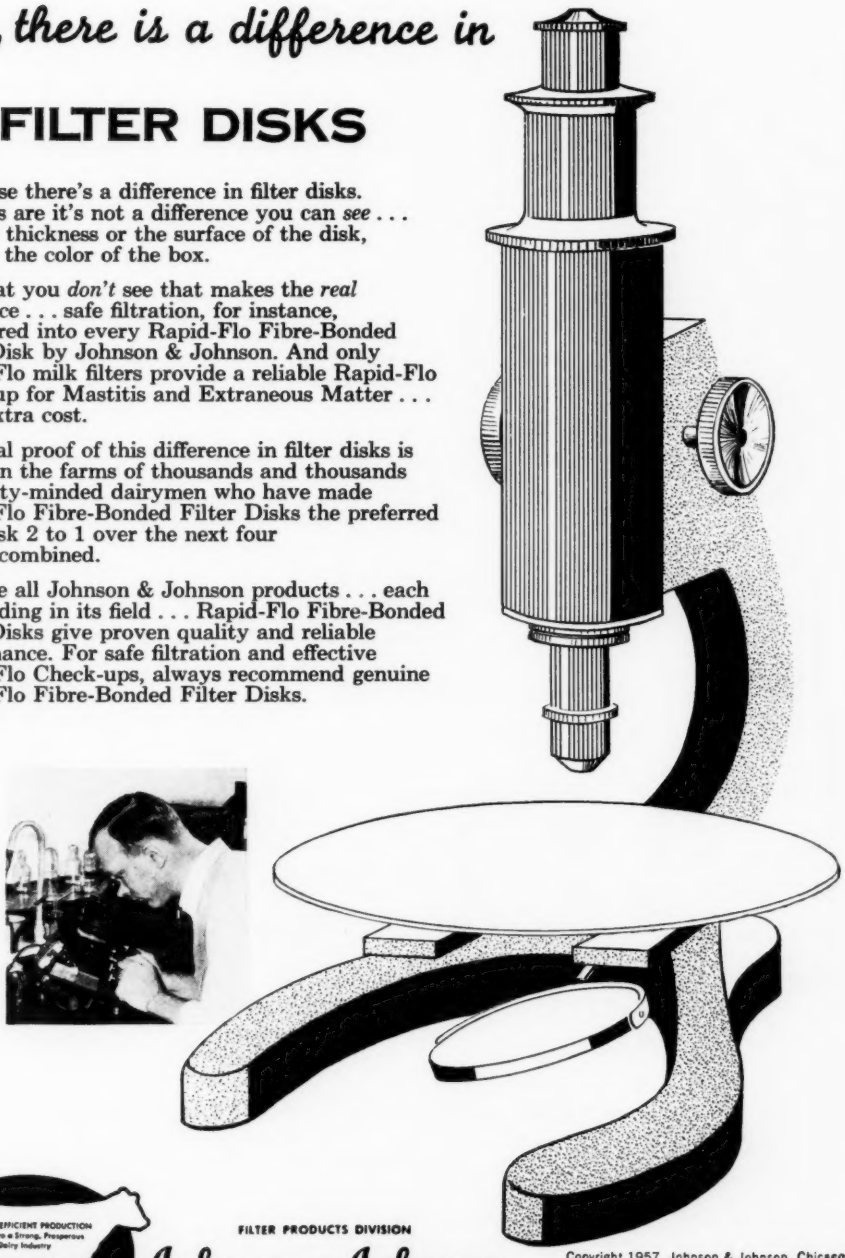

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Information prepared for *The Occupational Handbook*—scheduled for government publication this summer—shows that land-grant colleges and universities expect to supply 8,500 graduates in agricultural fields in 1957. (There are openings for 15,000.) These include 2,000 jobs in actual farming and ranching; 1,000 in agricultural research; 3,000 each in industry, business, and education; 500 in communications; 1,000 in conservation, and 1,500 in farm services.

Among the specific openings listed are agricultural extension service workers and vocational agricultural teachers; economists, finance workers and engineers, soil scientists and conservationists. Men also are needed to inspect, grade, and market farm products and to work with agencies preventing spread of plant pests, animal parasites and diseases.

National Milk Producers to Meet in Hartford

The fifth regional meeting of members of the National Milk Producers Federation will be held in Hartford, Conn., on April 26.

Dairy farmers and cooperative leaders from the New England and surrounding states are invited to the one-day meeting, to be held at the Hotel Statler. Discussions are planned covering pending national farm legislation, price-support programs, federal orders, production and sales outlook, and other subjects vital to the interest of the dairy farmer.

Guest speakers from the USDA planning to attend the meeting are H. L. Forest, director of the dairy division, Agricultural Marketing Service; Harlan J. Emery, chief of the program analysis branch of Commodity Stabilization Service dairy division, and Herbert C. Kriesel, head of the Agricultural Marketing Service dairy section, statistical and historical research branch.

Federation officials scheduled to participate are E. M. Norton, secretary, and J. P. Mason, economist.

Conference on Bulk Milk Handling to Be Held May 13-14 in Michigan

The National Conference on Bulk Milk Handling will be held May 13-14 at Michigan State Univ., East Lansing, under the joint sponsorship of the Depts. of Agricultural Engineering and Dairy Husbandry in cooperation with industry and federal and state governmental agencies.

During the two-day conference, the following topics will be discussed: Why Bulk Milk Handling?; Shifting from Cans to Bulk; Methods of Financing; The Dairy Producer and Bulk Tanks; Selecting the Tank; Frequency of Bulk Milk Pick-Up; Installation of the Bulk Milk Tank; Gauging and Testing Tanks; Pipeline Handling of Milk; 3-A Standards for the Tank

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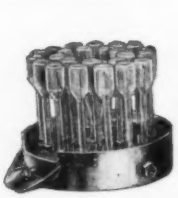

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Members of the national planning committee are D. B. FALCONER, vice-president, Detroit Creamery, Detroit, Mich.; L. C. PECKHAM, Milk and Food Program, U.S. Dept. of Health, Education, and Welfare, Chicago, Ill.; J. E. BULLIS, assistant vice-president, Carnation Company, Los Angeles, Calif.; C. O. DAVIS, editor-manager, *Milk Plant Monthly*, Kansas City, Mo.; F. M. SKIVER, chief, Dairy Division, Michigan Dept. of Agriculture, Lansing; D. L. MURRAY, Dairy Dept., Michigan State Univ., East Lansing, and C. W. HALL, M. S. U. Agricultural Engineering Dept.

Milk Shipment Meeting Held at Memphis

The Sixth National Conference on Interstate Milk Shipments was held April 23-25 at Memphis, Tenn.

The conference was highlighted by discussion of the following topics: Status of Use of the Program of Conference Agreements in Interstate Shipments of Milk; Report on the Fulfillment of Public Health Service Responsibilities Assigned by the Conference; Problems Within the Interstate Conference Agreements; Consideration of Conference Agreements by Task Forces on Regulations and Supervision, Certification Procedures, Utilization of Conference Policies, Channeling Information, and Laboratory Certification Procedures.

Did You Help?

The American Dairy Science Association membership campaign for 1957 is in its final phase. The goal of 2,200 members for this year is almost in sight. However, a few more membership prospects, and a few more members enrolled, are needed before our goal will be achieved. Have you done your part in this campaign? Did you suggest a membership prospect?

Too often we take things for granted. At a recent meeting of dairy leaders, we were surprised to learn through just casual conversation that two of the prominent leaders present were not members of the Association. Their memberships are now in the mail. Maybe you have had a similar experience. At the next dairy meeting or conference you attend, find out if the persons sitting to your right and left are members of, or would qualify for membership in, the American Dairy Science Association. They may become the members needed to reach our goal in 1957.

When you have the name and address of a prospective member, send them to the chairman of the Dairy Science Department of your State Agricultural College. This information will, in turn, be forwarded to the appropriate membership committee representative for action.

It should not be difficult to achieve, or even surpass, our membership goal if each member of the Association helps. If every member will suggest a member, the task should be finished within a short time. An adequate Association membership is a problem of great concern to each of us interested in the welfare and progress of the Dairy Industry. Did you help in the solution of this problem?

HAROLD E. CALBERT, *Chairman*
Membership Committee

Hoekstra Receives Award

WILLIAM GEORGE HOEKSTRA, assistant professor of biochemistry at the Univ. of Wisconsin, Madison, has been awarded a Merck Senior Postdoctoral Fellowship in the Natural Sciences for the academic year 1957-58. This fellowship, sponsored by Merck and Company, Inc., is administered by the National Academy of Sciences–National Research Council.

Dr. Hoekstra, who was born in Golden, Colorado, received the B. S. degree in 1950 from Colorado A & M; the M. S. and Ph.D. from the University of Wisconsin in 1952 and 1954, respectively.

Dr. Hoekstra plans to work on the metabolism and possible site of action of estrogenic compounds through the use of radioactive isotopes, and will carry out his research in the Isotope Section of the National Institute for Research in Dairying in Shinfield, England, under the direction of Dr. R. F. Glascock, an authority in this field.

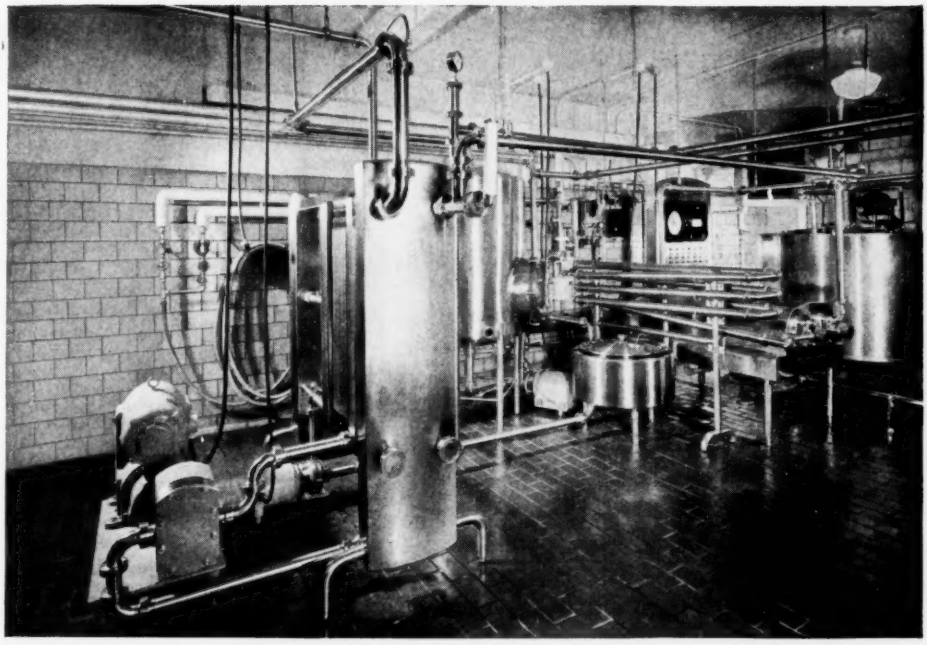
Editorial

P. A. WELLS and his group conducted for the second year a successful Research Conference on Milk Concentrates at the Eastern Utilization Research Branch, USDA, in Philadelphia, March 19-20.

Topics of a timely nature were discussed on the new phases of fundamental research which had a direct bearing on basic problems confronting the dairy industry.

Besides Dr. Wells, C. A. ZITTLE, M. J. PALLANSCH, D. SWERN, N. C. ACETO, and L. F. EDMUNDSEN of the Eastern Regional Laboratory, participated in the conference. H. E. CALBERT, Univ. of Wisconsin, Madison, discussed "Innovations in Improving Fresh Milk Concentrates"; and LEON TUMERMAN discussed recent developments in "Frozen Concentrated Milk."

Dr. Wells and his group did a real service to the dairy industry by sponsoring this con-



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ference, which dealt with problems at the fro tiers of learning. The specialized nature of th program appealed to experiment station an commercial research laboratory workers.

The organization and content of this pr gram could well serve as a pattern of planne performance for Dairy Departments over t nation to follow, and for the program com mittee of the manufacturing section for th annual meeting of A.D.S.A.—E. O. Herrel

Tjepkema Joins M & R Dietetic Labs.

Roy Tjepkema has joined the staff of th Research Dept. of M & R Dietetic Laboratori Inc., Columbus, O. Mr. Tjepkema graduat in dairy technology from the Univ. of Wisco sin, Madison, in 1952, and holds an M.S. degr from the same institution.

Salisbury to Attend Italian Meeting

G. W. Salisbury, head of the Dept. of Dai Science, Univ. of Illinois, Urbana, left f Milan, Italy on April 21 to take part the celebration of the 20th Anniversary of t founding of the Italian Experimental Ins tute "L. Spallanzani" in Milan, April 23 1957.

The Institute is named after Lazaro Sp lanzani, a famous 18th century Italian biolog Under the direction of Professor T. Bonador the Institute has been the center of Ital studies in mammalian reproduction and a ficial insemination.

Dr. Salisbury is to take part in a symposi of scientists from the Western European co tries, Canada, and the United States, deal with the physiology and metabolism of ma malian germ cells.

New Score Card Available from USD

A revision of the Dairy Farm Score C which was developed several years ago by t A.D.S.A. and the USDA has now been co pleted and has been printed by the Dairy Hu bandry Research Branch of the USDA.

Copies of the revised score card, "Dai Farm Sanitation Report," a "Report of t Regulatory Advisory Committee of A.D.S.A and an "Interpretation of Items" are all ava able in limited quantities from the USD Agricultural Research Service, Dairy Husban ry Branch, Beltsville, Md.

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STUDENT CHAPTER NEWS

A section devoted to the activities of dairy students

E. L. THOMAS, *Editor*

Cornell Dairy Students Assist with Cottage Cheese Clinic

F. V. KOSIKOWSKI, with the aid of the Cornell Dairy Science Association and the cheese manufacturing class (which, incidentally, was made up entirely of club members), sponsored a Cottage Cheese Clinic in November of 1956.

Some of the major advances of cottage cheese technology were discussed, including new methods for cottage cheese manufacture and a new formula for determining cottage cheese yield. There was a review of the problems inherent in quality control and merchandising of this type of cheese.

Two guest speakers on the two-day program were ERIK LUNDSTEDT, Consultant and Technical Advisor for H. P. Hood & Sons, Boston, and C. B. LANE, Manager and Research Director, Breakstone Brothers Company, Walton, N. Y. The equipment for demonstrations was

In this picture, Dr. F. V. Kosikowski holds a cheese knife, preparing to cut a vat of cottage cheese curd. Two students, Joseph Mathis (beside Kosikowski) and Donald Henry (in the foreground) are assisting.

lent by the Cherry-Burrell Corporation, United Metal Seal Company, and the Grace Machinery Company.

In the evening, the Dairy Science Club held a smoker for the men; refreshments consisted of a cheese board made up of twelve varieties of imported foreign cheeses.

Highlights of Kansas State College Dairy Club Activities

Service projects and activities have kept the Kansas State College Dairy Club busy this year, and more are planned for the spring semester. The Club held a "steak-fry" early in the fall for members, their friends, and the faculty, to furnish an opportunity for new students to become acquainted.

One of the purposes of the club is to be of service to the Dairy Department and dairy farmers of the state. With this in mind, the members provided lunch for visitors to the campus during the Kansas Artificial Breeding Service Unit Open House, Dairy Days at Farm and Home Week. and the annual Formula Feed Conference.

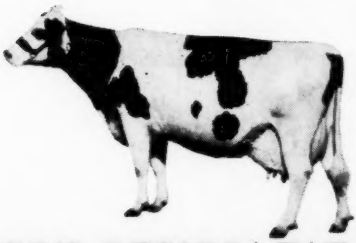
Club members were impressed with the success of the First Annual Dairy Club Banquet. It is hoped to make it an annual affair. This year, the honored guest was Dean C. W. MULLEN, long-time Assistant Dean, counselor, and friend of students in the School of Agriculture.

Another highlight of this semester will be the 29th Annual Little American Royal, a fitting and showing contest sponsored jointly by the Dairy Club and Block and Bridle Club. Later this spring, the Club will sponsor the college and FFA State Dairy Judging Contests. The year's activities will be climaxed at the spring "steak-fry," and members will be looking forward to summer vacation.

Minnesota Holds Winter Judging Contests

Again this year, four professional student clubs joined hands in sponsoring the annual Winter Judging Contests on the St. Paul campus of the Univ. of Minnesota. These contests, held over a three-week period, were sponsored by the clubs in the Animal Husbandry, Dairy, Poultry, and Agronomy departments. Winners were presented awards at a chicken barbecue sponsored by the Poultry Science Club on Februrary 14. ELTON KLAUSTERMEIER, a sophomore in Dairy Husbandry, was named top judge among those participating in all contests. He was also top over-all judge in Dairy Cattle and Dairy Products judging. Awards in the form of trophies and other appropriate objects were donated by various trade associations, concerns, and individuals representing the fields served by the participating departments. Also honored at this year's Awards Program were members of the 1956, 1946, 1936, and 1926 judging teams.

At its March meeting, the Dairy Science Club heard an interesting and informative discussion by W. E. PETERSEN, regarding research on "Protective Milk."



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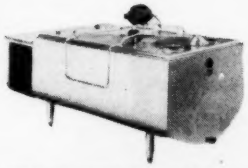

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Mississippi State Has Huffman as Speaker

The Mississippi State Dairy Club, a Student Affiliate Chapter of the A.D.S.A., held its annual student and alumni banquet in January in the Blue Room of the College Grill. Many former students returned for the occasion. Each senior student was called upon to introduce another member of his class, which brought forth many humorous situations not too generally known previously.

Featured in the program was the presentation of awards. The $300 Borden Award was presented to JAMES G. HAMILL, by W. E. SCHMIDT of the Borden Food Products Company. Mr. Hamill was the recipient also of the Ralston-Purina Scholarship. The Higgins Awards, consisting of a $200 and a $100 scholarship, were presented to C. E. MANNING and JAMES W. ROWZEE by JOHN BETTERSWORTH.

The guest speaker for the evening was C. F. HUFFMAN, Michigan State Univ., East Lansing, president of A.D.S.A., who had participated in the Dairy Fieldmen's Short Course. Dr. Huffman made a very informative talk on opportunities for the dairy graduate, as well as on the activities of the A.D.S.A.

Active North Carolina State College Club Joins with A.D.S.A.

The Dairy Science Club at N. C. State College has just recently become a student chapter of the A.D.S.A. It had, however, been very active prior to joining the parent organization. All of its activities are carried out in conjunction with the Animal Industry Club, which is composed of members from both the Dairy Science and Block and Bridle Clubs.

The biggest undertakings came during State Fair in October, 1956. The Club members showed livestock from the State College herds and operated the new dairy bar on the State Fairgrounds. The Club won premium breeder in the Holstein show and premium exhibitor in the Ayrshire show.

Close contact is maintained between the Club and the livestock men of North Carolina by holding several barbecues at livestock meetings on the campus. On October 17, the Club provided a free barbecue for all breeders exhibiting livestock at the North Carolina State Fair. At the annual Dairymen's Conference, 436 plates of barbecue were sold. Providing meals for other meetings, such as Beef Cattlemen's Conference, was continued this year.

The money earned by the Club is being spent to help defray the expenses of members of the various judging teams (Dairy, Dairy Products, Livestock, and Meats). A scholarship fund is being established for a deserving student of Animal Industry. A bus will be chartered by the Club for three days to visit North

Carolina livestock farms. An annual steak dinner, to be held May 17, for the Animal Industry staff, students, and their guests, will be partially financed by the Club.

O.S.U. Student Club News

THOMAS W. KUNKEL of Euclid, Ohio, a senior in Dairy Science, was awarded the S. M. Salisbury Memorial Scholarship for 1957. The Salisbury Scholarship Fund has been developed in memory of Prof. SCHYLER M. SALISBURY, who was a member of the Dairy Science staff for over 30 years. The contributions of Ohio State Univ. alumni and friends of the late S. M. Salisbury have established this fund. In selecting the recipient of this scholarship, the committee considers integrity, interest in dairy science, scholastic ability, need of student, and extra-curricular activities.

Thomas W. Kunkel

The Junior A.D.S.A.'s Annual Winter Dinner-Dance was held in the west ballroom of the Ohio Union, Saturday, February 16. The 150 people who attended the dinner-dance enjoyed a delicious meal preceding the program.

The annual election of officers for the Junior A.D.S.A. was held at the March 7 meeting. Elected were: RONALD EBERHARD, president; HAROLD SWAISGOOD, vice-president; THOMAS SETTLEMIRE, treasurer, RICHARD GITHENS, secretary.

The Production section is sponsoring the first Buckeye Dairy Show on April 20, in connection with Purebred Dairy Cattle Association's judging school, which will be held at the Ohio State Univ. on April 19 and 20.

Rutgers Univ. Dairy Science Club Activities

The Dairy Science Club of Rutgers Univ., New Brunswick, N. J., sponsored two luncheons again this year. The first was for the Cheese Manufacturers' Conference on January 18; the second, for the Ice Cream Manufacturers' Conference, January 25. By serving these two groups, the Club earned approximately $75 for its treasury.

During the month of December, the Club published its annual Christmas Letter; its editor-in-chief was JAMES THOMPSON. This letter is sent each year to the club members and club alumni, telling them what the Club has accomplished the past year and what is new in the department—especially in the way of research.

Babcock Dairy Science Club to Be Active at Dairy Manufacturers' Conference

The February meeting of the Babcock Dairy Science Club was held, with 25 members present. One of the chief means of making money for the Club was selling refreshments at the annual Dairy Manufacturers' Conference, held at the Univ. of Wisconsin, Madison, April 2 and 3. Plans and committees were again set up this year for carrying out this project. Also, the club printed a paper to be distributed to all those attending the Conference. This paper included news about the Club and its activities, the Univ. of Wisconsin Dairy Products Judging Team, and also gave some information about the seniors graduating in the Dairy Industry Dept. It is hoped that, with this information, interviews will be set up between graduates and prospective employers.

The speaker for the evening was MYRON DEAN, of the Dairy and Food Industries Dept., who spoke on the advantages to be gained from participation in the dairy products judging work.

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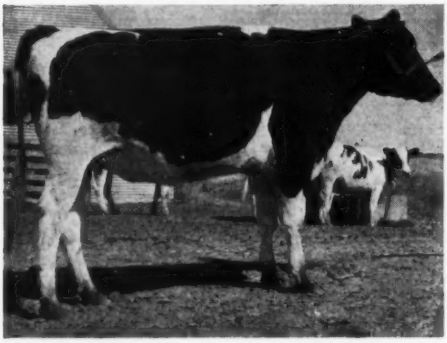
Washington State Dairy Club Reports Projects for Financing Judging Team Trips

Heifers and vending machines were the main topics for discussion at the February meeting of the WSC Dairy Club.

Last fall, the Dairy Club consigned a bred, registered Holstein heifer to the Washington State Holstein Sale at Mount Vernon, Wash. The heifer brought $450, which was above the sale-average. With the profits from this heifer, the Dairy Club purchased three more registered Holstein heifers. These heifers have been bred to the State College of Washington's popular herd sire, Carnation High-Lighter. The heifer committee reported that the heifers will be consigned to the Washington State Holstein Spring Sale, to be held in Spokane the latter part of April. The money realized from their sale will be used to purchase another heifer for the annual fall sale. The increased scope of this project will enable the Club to finance the dairy judging team trips to the National Dairy Congress at Waterloo, Ia., the International Livestock Exposition at Chicago, and the Grand National Exposition at San Francisco.



Registered Holstein heifer consigned by Washington State Dairy Club to recent sale.

The vending machine committee reported a similar increase in the scope of their project. The Dairy Club rents vending machines from the Department of Dairy Science. These machines are placed in dormitories and other living places for the students' convenience. Bottled milk and ice cream bars are quite popular at WSC.

The Dairy Club pays one of its members to service and maintain the machines. Profits are used to finance the Dairy Products Judging Team to the International Dairy Products Judging Contest, which is held at Atlantic City, N. J., every other year.

The WSC Dairy Club can think of no better way to promote the dairy industry than by selling heifers, milk, and milk products.

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EFFECT OF POTASSIUM OROTATE AND METHIONINE SUPPLEMENTATION ON FEED CONSUMPTION AND GROWTH OF DAIRY HEIFERS[1]

J. M. WING

Department of Dairy Science, Florida Agricultural Experiment Station, Gainesville

Jersey heifers fed rations supplemented with methionine and potassium orotate made more economical gains than those without the supplements. *Editor.*

Orotic acid is a growth factor for bacteria *(2)*, laboratory animals *(3, 4)*, and, with methionine, for young dairy calves *(8)*. Its mode of action, however, is not clear. Since previous investigations were limited to small calves and monogastric species, possibly orotic acid is, or substitutes for, a normal rumen product. The present experiment was designed to determine whether or not potassium orotate with methionine affects growth and feed consumption in dairy heifers with well-established rumen functions.

EXPERIMENTAL PROCEDURE

Twelve Jersey heifers, averaging nine months of age, were divided into two comparable groups. All subjects were confined to individual dry lots and were provided shade, water, hay, concentrate feeds, and mineral supplements.

Roughage digestibility could be an important factor, since rumen microorganisms might be stimulated by the supplements used. For this reason, the hay selected was of poor quality (of advanced maturity and produced under unfavorable curing conditions). Pangola grass hay was fed the first month and, thereafter, Alyceclover hay. Both hays were fed free choice. A maximum of five pounds per head daily of a concentrate feed designed to be rich in naturally occurring orotic acid was offered. Composition of the concentrate mixture was as follows:

Ground shelled corn	30 parts
Ground oats	30 parts
41% cottonseed meal	20 parts
Nonfat dry milk solids	20 parts
Bone meal	2 parts
Salt	1 part

Received for publication August 1, 1956.

[1] Florida Agricultural Experiment Station, Journal Series No. 504.

The experimental ration contained additional potassium orotate and methionine in the amount of 272 g. of each, per ton of concentrate feed. Individual feed consumption was recorded daily throughout the three-month period. Body weight (two-day average) was determined initially and at one-month intervals.

RESULTS AND DISCUSSION

A reduction in appetite, and general unthriftiness, were apparent in the treated group by the end of the second week. Fecal examination showed the presence of roundworms in all subjects, though they appeared to be more numerous in heifers that received the supplements.

The initial inferiority of the treated group might be attributed to stimulation of internal parasites by the potassium orotate and methionine.

All animals were drenched with 2 oz. of phenothiazine, and all improved in appearance. There were no symptoms of parasite infestation by the end of the first month, and little, if any, difference in appearance between groups.

By the end of the second month, although health was excellent in all subjects, supplemented heifers exhibited glossier coats and were more alert in appearance than were the controls.

Heifers that received the supplements gained an average of 116 lb. (Figure 1) compared with a group-mean gain of 100 lb. in controls and an expected gain of approximately 90 lb. *(6)*. This difference was statistically significant at $P = 0.05$.

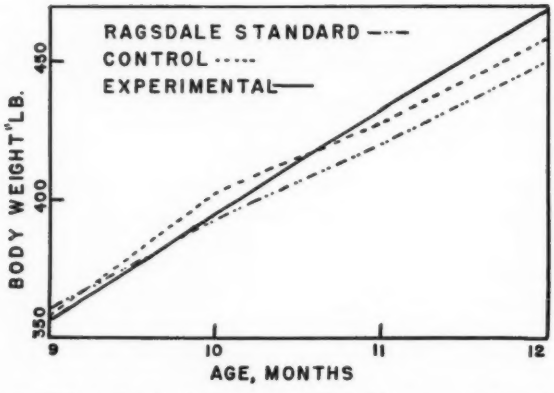

FIG. 1. Effect of potassium orotate and methionine on body weight.

The experimental group was superior in efficiency of feed utilization, requiring 3.1 lb. of TDN per pound of gain, exclusive of maintenance, as compared to 3.5 lb. for controls. This difference, likewise, was significant at $P = 0.05$. No appreciable difference was observed in appetite of this group for either the hay or the concentrate mixture.

Reservations must be made in evaluating these data, however, because both TDN content of the feed *(5)* and maintenance requirements *(1)* were calculated.

The rate of 272 g. of each supplement per ton was based on results of earlier work *(8)*, the assumption that nonfat dry milk contains 0.6 mg. of orotic acid per gram *(7)*, and that animal requirements vary with the 0.73 power of body weight *(1)*. There is no conclusive proof that other levels would not be more effective. The fact that less response was observed in heifers than in small calves *(8)* could be the result of suboptimum intake by the large animals, as well as of differences in rumen physiology and stimulation of internal parasites.

Results are similar to earlier data, which indicate a synergism between orotic acid and methionine *(8)*. This synergism recently was confirmed with mice *(4)*.

If orotic acid is synthesized in the rumen, a suboptimum amount apparently was produced under the conditions of this experiment. The amount of orotic acid supplied by skimmilk in the concentrate mixture likewise was insufficient for best results. It seems possible, however, that the effect of supplementation was partially obscured by the orotic acid content of skimmilk solids in the concentrate feed.

SUMMARY

Between the ages of nine and 12 months, six Jersey heifers which received concentrate feed containing 272 g. each of supplementary methionine and potassium orotate gained an average of 115 lb. and required 3.1 lb. TDN per pound gained. The average gain of a control group was 100 lb., with a feed efficiency of 3.5 lb. of TDN per lb. of gain. Both observations were statistically significant at $P = 0.05$. Increased activity of internal parasites was indicated in the treated group.

ACKNOWLEDGMENTS

The author gratefully acknowledges the cooperation of J. P. Boggs, who fed and managed the animals. Thanks are also extended to Specifide, Inc., Indianapolis, for the methionine and potassium orotate used in this work.

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TECHNIQUE FOR IN VIVO MEASUREMENT OF THYROIDAL I^{131} IN CATTLE [1, 2]

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Quantitative changes in thyroidal I^{131} can be measured with a sensitive scintillation counter, mounted on a flexible arm which is attached to a holder that immobilizes the neck and head of the animal. One gram of thiouracil per 100 lb. inhibits reutilization of I^{131} from metabolized thyroid hormone. The daily secretion rate of individual animals can be measured in terms of L-thyroxine. *Editor.*

The rate of secretion of the thyroid hormones plays an important role in the growth process and in the maintenance of intense milk secretion in dairy cattle. In the absence of the thyroid glands or in hypothyroidism the growth rate is slowed, and milk may be reduced from 30 to 40 per cent. Conversely, with optimal levels of thyroid hormone secretion, the growth rate is accelerated, and the intensity of milk secretion is increased to the point where other hormones become limiting factors in productivity *(21)*.

At the present time, the selection and mating of dairy cattle are based upon the total production of milk and fat in a lactation period of 305 or 365 days. Information has not been available as to secretion rates of the various endocrine glands upon which the total productivity of the animals is dependent. As a consequence, breeders of dairy cattle have not known why certain individual cows or the progeny of certain sires are incapable of measuring up to high standards of production.

As a result of the intense endocrine research of the past 25 years, the role of the various hormones in stimulating growth of the udder and the intensity of milk secretion have been determined *(20)*. There are two methods by which the activity of the individual endocrine glands may be measured.

One method consists of administering exogenous hormone and observing the effect of it on the intensity of milk secretion. If the milk yield increases during the period of administration, it may be inferred that the rate of secretion of the hormone by the cow is a limiting factor in its productivity. If the cow had inherited a higher secretion rate for the given hormone, its productivity would have been equal to that stimulated by the additional hormone *(21)*.

Received for publication September 6, 1956.

[1] Contribution from the Missouri Agricultural Experiment Station, Journal Series No. 1663. Approved by the Director.

[2] Aided in part by a grant from the U. S. Atomic Energy Commission [Contract No. AT (11-1) -301].

[3] The authors are indebted to C. J. Bryan for technical assistance throughout the experiments described.

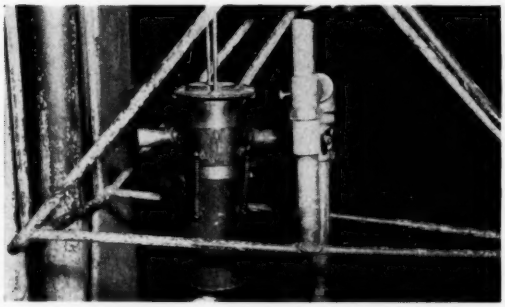

FIG. 1. Scintillation counter and flexible arm in position for operation.

The second method of determining valuable information about the inherited rate of secretion of the important endocrine glands is direct measurement of the rate without respect to other endocrine glands.

In a series of earlier reports from this station *(18)*, a method was reported by which the average thyroxine secretion rate of experimental animals and fowls was estimated, using a goitrogen to block thyroxine secretion and administering graded doses of thyroxine. By this technique, it was necessary to sacrifice the animals and determine the weight of the thyroid gland. Such a method is not applicable to the larger domestic animals, owing to their high value. Furthermore, the method determined the average thyroxine secretion rate rather than the secretion rate of individual animals.

In the development of alternative methods of determining the thyroxine secretion rate, study was devoted to the blood levels of the thyroxine-like I^{131} and total I^{131}. This method involved the serial sampling of the blood with its attendant disturbance of the animal. While the blood methods have contributed greatly to our understanding of thyroid physiology in the rat *(16)*, in the fowl *(3)*, and in cattle *(15)*, a greatly improved method has been developed in our laboratory for obtaining highly reproducible in vivo determinations of thyroidal I^{131} in cattle. The object of this paper is to report details of this new method and present some preliminary observations upon its usefulness in a study of thyroid function in cattle.

Equipment development. Several investigators have developed methods for determination of thyroidal I^{131} in laboratory animals *(4, 10, 11, 12)*. Extensive investigations have been made in devising methods for measurement of I^{131} in the thyroid gland of the human *(1, 2, 4, 8)*. Fundamentally, the latter methods consist of locating one or more receptors (GM tubes or scintillation counters) at approximately 30 cm. from the thyroid gland. Perlmutter *et al. (14)* have shown that at a distance of 30 cm. between the radioactive source and the receptor, a variation in location of 1 cm. produced a mean of three per cent difference, compared with a mean of 42% difference at a distance of 1 cm. The inverse-square law, i. e., the intensity of radiation, is inversely proportional to the square of the distance between the source of radiation and the point of measure-

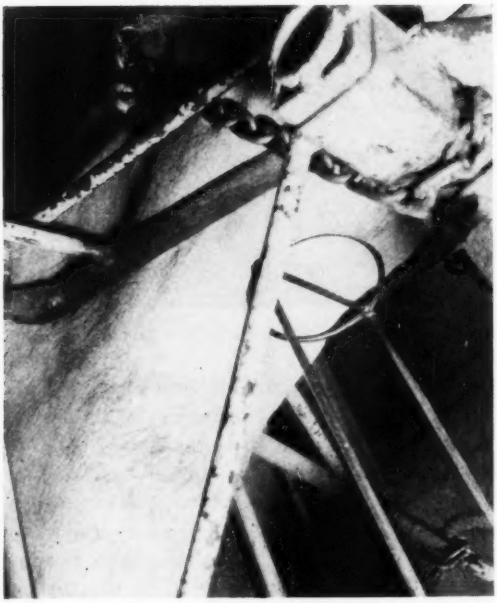

FIG. 2. Spacer and steel ring located over the thyroid gland.

ment, indicating that precision measurements of thyroidal I^{131} should be made at as great a distance as the sensitivity of the detector will permit.

A. Equipment for restraining the animal. Since the distance between the thyroid gland and the receptor must remain constant during the period of measurement, the head and neck of the animals were immobilized with a Ranger Speed table.[4] This head-holder was attached to a Ranger Cattle Chute (Figure 3) or attached to the metal upright bars of a cattle stall (Figure 4). The head-holder was attached to the chute at a point 31 in. above the floor. Portions of the head-holder directly below the throat were removed, to allow access to the thyroid region.

After a short training period, dairy cows and calves can be handled in this apparatus without exciting them. Dairy animals that are trained to lead can be handled with the head-holder attached to a cattle stall. When handling beef animals or untrained dairy animals, restraint in a cattle chute is required during the training period.

B. Radiation-Detecting Equipment. A scintillation counter was employed, since these instruments are 30 to 50 times more efficient than the GM tube. This counter contained a 1″ x 1″ NaI (thallium-activated) crystal, and was connected by a 20-ft. coaxial cable to a combination scaler and analytical rate meter.

The head-holder was modified to permit the attachment of a scintillation counter-holder on a flexible arm. The flexible arm allowed adjustment of the

[4] Delphi Products Co., Delphi, Indiana.

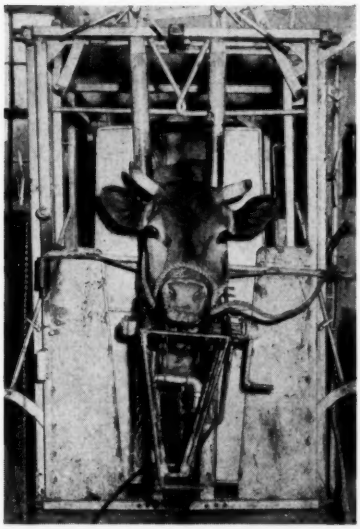

FIG. 3. In vivo determination of thyroidal I^{131} in the cow.

counter in vertical and horizontal positions under the thyroid regions of the throat of the animal.

In order to develop a sturdy spacer, two steel rods 6 mm. in diameter and 30 cm. in length were attached to the scintillation counter-holder. The upper end of the steel rods terminated in a steel ring 10½ cm. in diameter, which was rein-

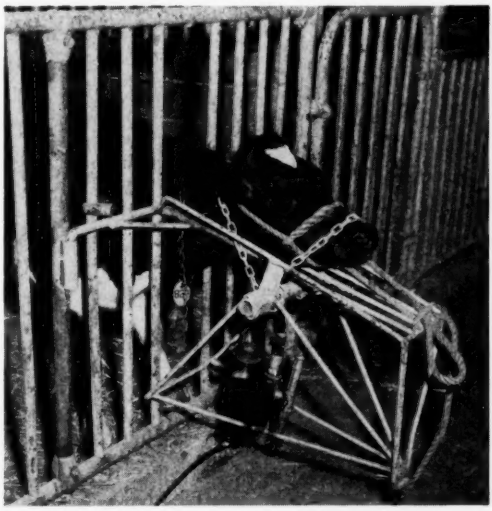

FIG. 4. Scintillation counter and head-holder attached to a cattle stall.

forced by a bisecting steel rod (Figure 2). A spring balance was inserted between the counter-holder and the flexible arm to allow the adjustment of uniform pressure between throat and spacer (Figure 1). This device minimized the effect of variable dewlap size and allowed reproducible geometry.

In operation, the front of the steel spacer ring was placed at the posterior aspect of the cricoid cartilage. The spacer ring in this position covered the first two or three tracheal rings. These anatomical landmarks in the region of the thyroid gland are easily located by palpation of the neck. Maximum counting rates were consistently observed in this region.

EXPERIMENTAL METHODS

The animals available for the preliminary study were dairy and dwarf beef cattle, fed and housed in a unit separate from the University herd. All animals were allowed access to iodized salt during the experimental period.

From 100 to 300 μc. of carrier-free radioiodine (NaI^{131}) were injected intravenously into each animal at the beginning of the experimental period, the amount injected varying with the duration of the experiment. Results have been expressed in terms of the percentage of the injected dose. Corrections were made for the radioactive decay of I^{131}. Corrections were not made for absorption of gamma radiation by the neck or the spacer ring, since these corrections were assumed to be constant for each individual animal throughout the experiments. Backscatter was virtually eliminated by adjustment of the discriminator circuit to reject low-energy radiation.

Experiment I, Uptake and Release of Thyroidal I^{131}. Three Jersey cows, ranging in weight from 900 to 1,200 lb., were injected with carrier-free NaI^{131}, and measurements of thyroidal I^{131} were made daily with the described equipment for 14 days. Maximum observed uptake of I^{131} by the bovine thyroid varied from 48 to 72 hours after injection. After this period of time, the release of I^{131} as thyroid hormone exceeded the rate of uptake, and thyroidal radioactivity began exponentially to decline.

In many ways the decline in radioactivity of the thyroid gland is similar to the decline in milk yield with advancement of the lactation period. Both appear to indicate first-order reactions where the rate of reaction is directly proportional to the concentration of the reacting substance.

Brody, Ragsdale, and Turner *(6)* have demonstrated that milk secretion during the declining phase of lactation can be predicted according to the equation:

$$Mt = Mo\, e^{-kt} \qquad (1)$$

where Mt = milk production during any month, t; Mo, the theoretical value of the milk production at the time of parturition; e, the base of natural logarithms, and k the rate of decline of milk production.

The amount of I^{131} remaining in the thyroid gland can be predicted by a similar equation:

$$I_t^{131} = U\, e^{-kt} \qquad (2)$$

where I_t^{131} is the per cent of injected dose corrected for radioactive decay re-

maining in the thyroid on any day, t; U is the theoretical maximum uptake of I^{131} by the thyroid at zero time expressed in per cent of injected dose; e, the base of natural logarithms; k is the rate constant indicating the daily disappearance of I^{131} from the thyroid gland, and t is time in days.

The decline in radioactivity of the thyroid gland is primarily dependent upon the rate of release of thyroid hormone and secondarily dependent upon uptake rate of I^{131} from metabolized thyroid hormone. When I^{131}-tagged thyroxine is released into the blood, it is then metabolized in various cells of the body and I^{131} is removed from the carbon skeleton of the thyroxine molecule. As a result of this process, I^{131} again becomes available to the thyroid and the kidney. It is generally assumed that such I^{131} is collected by the thyroid in exactly the same manner as the initial injection of I^{131}. This process is called "recycling" or "reutilization" of iodine. The true rate of release of thyroid hormone is masked to the extent and degree that recycling of I^{131} occurs.

In order to separate these two processes, the techniques and terminology of Brownell *(7)* were employed. The k'_4 values were based upon the calculated slope of the regression line indicating the net release of thyroidal I^{131}. These values were stated in terms of hours; whereas, the k value in equation (2) was stated in days. The k'_4 values are much lower than the true value, because I^{131} from metabolized thyroid hormone is picked up and reutilized by the thyroid. It is generally assumed that I^{131} is reutilized to the same degree that the original I^{131} was utilized by the thyroid gland. Therefore, the equation of Brownell

$$k_4 = \frac{k'_4}{1 - U} \tag{3}$$

where k_4 is the true rate constant for release of thyroid hormone, k'_4 is the observed rate of release of thyroidal I^{131}, and U is the theoretical uptake of I^{131} (decimal fraction of injected dose), obtained by extrapolation to zero time, was employed.

These calculations are necessary because animals may have the same rate of hormone release (k'_4) while reutilization is occurring to a much greater degree in those with a high uptake (U) than in those with a low uptake.

If the k'_4 values were equal, then the animals with lower I^{131} uptake would have a lower rate of release (k_4) than those with a higher uptake.

Representative rate constants for hormone release (k_4) and percentage uptake of injected dose are presented (Table 1).

When regression lines are fitted by the method of least squares to data obtained during the release phase, daily measurements follow very closely to the calculated values. Day-to-day average temperature variations (19° to 40°) apparently did not affect daily measurements (Figure 5).

TABLE 1

Thyroid function of dairy cattle

Cow No.	U (% Dose)	k_4 (release rate)
310	57.1	.0016 ± .0004
160	44.6	.0038 ± .0006
544	38.2	.0082 ± .0006

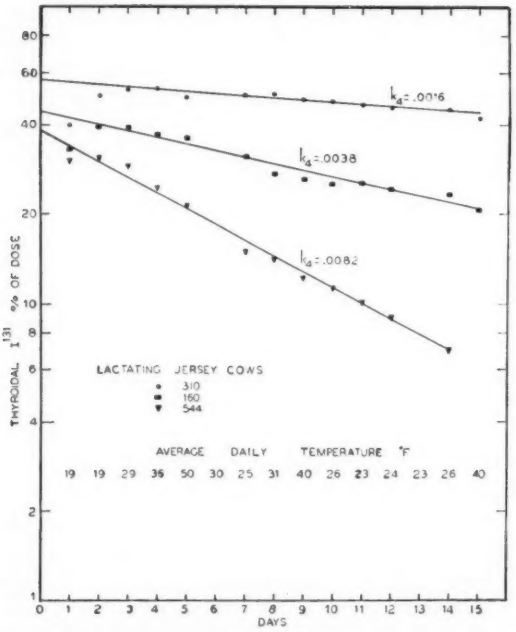

FIG. 5. The observed rates of release of thyroidal I^{131} in the bovine. (k_4 values are rate constants corrected for reutilization.)

Standard errors were calculated from the deviations of daily measurements from the calculated slope. Those standard errors were applied to the k_4 values. Standard errors were apparently quite low when compared with the data of Blincoe and Brody *(5)*. Wide variations (.0016 to .0082) were found to exist in k_4 values between dairy cows.

In order to investigate the rate of release of thyroidal I^{131} uncomplicated by recycling, experiments were undertaken to block the recycling of I^{131} through the thyroid gland.

Experiment II, Effect of Thiouracil on Rate of Release of Thyroidal I^{131}. When goitrogens such as thiouracil or 6-methyl thiouracil are administered to animals, the ability of the thyroid to reutilize I^{131} is inhibited. While the thyroid can pick up iodine under these conditions, it is unable to convert iodine to thyroid hormone, and loses such iodine rapidly to the blood *(1)*. During this process the thyroid continues to secrete preformed hormone. When reutilization is blocked, the rate of release of thyroid hormone may present a better parameter of thyroid function, since recycling of I^{131} does not complicate the situation.

In this experiment, four Jersey cows were injected with I^{131} and daily measurements of thyroidal I^{131} were made for 13 days, to determine the normal rate of uptake and release. On the 13th day thiouracil was administered at approximately 1 g. per 100 lb. body weight by gelatin capsule. Daily measurements

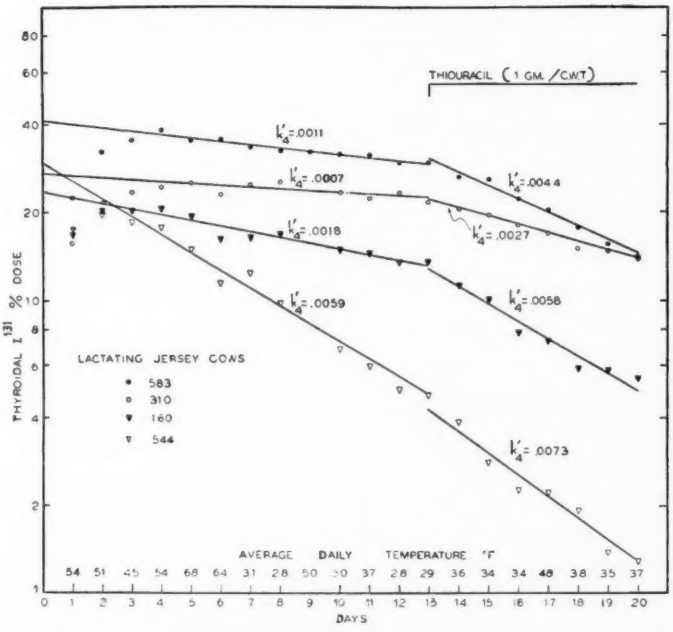

FIG. 6. The effect of thiouracil on the apparent rate of release of thyroidal I^{131}. (k'_4 values are rate constants uncorrected for reutilization of I^{131} by the thyroid gland.)

of thyroidal I^{131} and administration of thiouracil were continued for seven days. The net rate of release (k'_4) was calculated for each period (Figure 6). Within 24 hours after the administration of thiouracil the apparent rate of release of thyroidal I^{131} increased, and a new steady state of loss of thyroidal I^{131} was observed. Changes in the release rate are shown (Table 2 and Figure 6).

The k_4 value, as well as the k'_4 value, under thiouracil furnishes information of physiological importance. When these values are high they indicate that functional activity (thyroid secretion) is high. On the other hand, when these indices are low, it is expected that the animals will be poor-growing and poor-lactating animals, owing to low thyroid function. However, these measurements do not provide a direct estimate of the actual amount of hormone produced, but only a measure of relative rates of thyroid function.

TABLE 2

Effect of thiouracil on thyroid function

Cow No.	k'_4 (Control period)	k'_4 (Thiouracil period)
583	.0011 ± .0003	.0044 ± .0004
310	.0007 ± .0004	.0027 ± .0004
160	.0018 ± .0003	.0058 ± .0005
544	.0059 ± .0012	.0073 ± .0008

Experiment III, Determination of the L-*thyroxine Secretion Rate.* The thyroxine secretion rate of an animal is controlled by the interplay of a hormone secreted by the anterior pituitary lobe, called thyrotropin, and the thyroid gland and its secretion of thyroxine. When thyrotropin is secreted in increased amounts it stimulates the thyroid gland to increased thyroxine production. The secreted thyroxine in turn acts upon the pituitary, to depress the secretion of thyrotropin. Thus, the variation in thyroxine secretion in cattle is dependent upon the level of interplay between the two hormones. In cattle with a high thyroxine secretion rate, a larger amount of circulating thyroxine is required to depress the secretion of thyrotropin.

This normal equilibrium between the two hormones makes possible the determination of normal L-thyroxine secretion rate of cattle. If an amount of thyroxine which equals the thyroxine secretion rate of the normal animal is injected, then the secretion and discharge of thyrotropin by the pituitary is stopped; this in turn stops the secretion and discharge of thyroxine by the thyroid gland. When this occurs, the discharge of I^{131}-tagged thyroxine is inhibited.

Three heifers were injected with I^{131} and after 48 hours daily measurements of thyroidal I^{131} were made for a period of four days. After this time thiouracil was administered at the rate of 1 g. per 100 lb. body weight. As in the previous experiment, a prompt change in the apparent rate of release was observed during the next three days. At the end of this period the animals were injected with increasing doses of L-thyroxine during periods of three days each, and the administration of thiouracil was continued. Measurements of thyroidal radioactivity were made at the end of each three-day period (Figure 7). As thyroxine injections were progressively increased, the secretion of thyrotropin was progressively decreased. In turn, the discharge of I^{131}-tagged thyroid hormone was inhibited, as shown by the reduced rate of loss of thyroidal I^{131}. At the level of thyroxine which completely blocked the apparent release of thyroidal I^{131}, equilibrium was assumed to be obtained (Figure 7). This is tentatively assumed to represent the equivalent of the animal's L-thyroxine secretion rate.

DISCUSSION

Equipment has been described that makes possible a quantitative study of changes of thyroidal I^{131} in the bovine, utilizing a scintillation counting system. This equipment permits immobilization of the head of the animal and location of the scintillation counter at 30 cm. from the thyroid, for a sufficient period of time to permit measurements of high statistical significance.

Three criteria of thyroid function in dairy cattle have been suggested, utilizing the described equipment:

(1) The relative rate of release of thyroid hormone (k_4) corrected for recycling according to Brownell's equation. (2) The k'_4 value when recycling of I^{131} is blocked by use of goitrogens. Stanbury *et al. (19)* have suggested that inorganic iodine may be lost from the thyroid during goitrogen treatment. However, the present investigations have shown that thyroxine will inhibit the loss of I^{131} from the thyroid during thiouracil administration. It would appear more

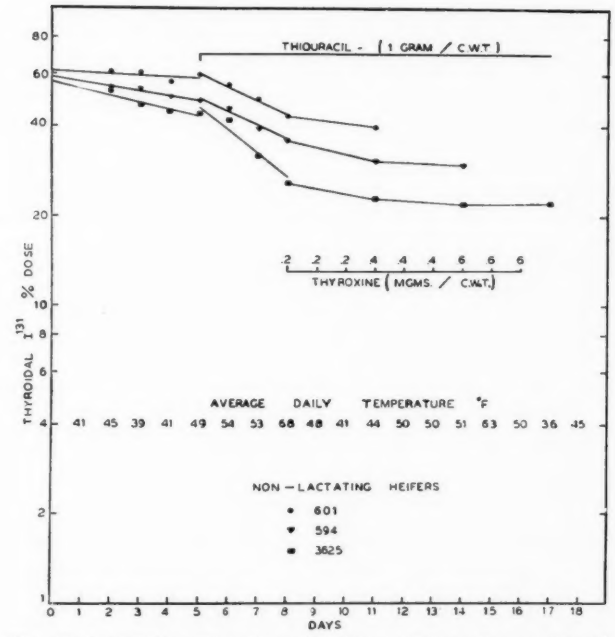
THIOURACIL - (1 GRAM / C.W.T.)
THYROXINE (MGMS. / C.W.T.)
2 2 2 4 4 4 6 6 6
THYROIDAL I^{131} % DOSE
AVERAGE DAILY TEMPERATURE °F
41 45 39 41 49 54 53 68 48 41 44 50 50 51 63 50 36 45
NON-LACTATING HEIFERS
601
594
3625
DAYS

FIG. 7. The effect of thiouracil and graded doses of thyroxine on thyroid function.

logical to assume that the reutilization corrections suggested by Brownell *(7)* do not completely fit the experimental model. (3) Replacement therapy techniques during thiouracil administration can be used as an index of thyroid function. Although these data do not prove that inorganic I^{131} is not released in association with the release of I^{131}-tagged thyroxine, they indicate that in the absence of the release of I^{131}-tagged thyroxine and during the treatment with goitrogens inorganic I^{131} is not released (Figure 7).

Replacement techniques have been applied to the rat *(17)*, to the rabbit *(9)*, and to sheep *(13)* and the fundamental approach is believed to be sound; but due to much slower rate processes in large animals, more investigation is needed on a number of time relationships, involving the mechanism of the pituitary-thyroid system as well as the biological half-life of thyroxine in the cow. A discussion of these problems will be reserved for later presentation.

SUMMARY

Equipment has been described which makes possible the quantitative study of thyroidal I^{131} in the bovine. This equipment consists of a scintillation counter mounted on a flexible arm, which is attached to a head-holder designed to immobilize the head and neck of the animal. Sufficient sensitivity is provided by the counting system to permit the use of 50 or less μc. of I^{131}.

Data have been presented on the normal uptake and release of I^{131} by the bovine thyroid and the effect of goitrogens upon reutilization of I^{131} from metabo-

lized thyroid hormone has been described. One gram of thiouracil per 100 pounds has been found to inhibit reutilization.

Replacement therapy with thyroxine during thiouracil treatment has indicated the feasibility of measuring the actual daily secretion rate of individual animals in terms of L-thyroxine.

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SOME FACTORS AFFECTING THE DETERMINATION OF PLASMA PROTEIN-BOUND IODINE, USING THE ALKALINE FUSION–CERIC SULFATE METHOD [1]

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Blood plasma should be processed and assayed for protein-bound iodine immediately after it is collected, to avoid the interaction effects of length and temperature of storage with each other and with animal. *Editor.*

The determination of plasma protein-bound iodine (PBI) by the alkaline fusion–ceric sulfate method *(2, 3)* has been proposed as a simplification of older iodine distillation procedures *(1, 4, 7)*. Few data have been presented that show the sources of experimental error in the use of the alkaline fusion method. This paper presents a study of the effect of the length and temperature of storage of bovine plasma on plasma PBI values, together with an analysis of error term variance as contributed by fusions and by replicate determinations within fusions. Also presented is a method of calculating plasma PBI values, whereby per cent transmittance readings, iodine standards, and simple proportionality calculations may be used.

METHODS AND PROCEDURES

Blood for this study was collected from the jugular vein of each of four dairy cows (three Holsteins and one Guernsey) which were of similar age and stage of lactation. The blood was centrifuged immediately to obtain the plasma. An anticoagulant consisting of 24 g. of ammonium oxalate, 16 g. of potassium oxalate, and 1 liter of water was used (10 ml. for 100 ml. of blood). The anticoagulant was evaporated to dryness in the blood-collection tubes.

A factorial design was used in these studies in which duplicate 1-ml. subsamples were prepared for fusion per subtreatment, by pipetting plasma into Pyrex ashing tubes. The subsamples were then stored at temperatures of −20, 2, and 25° C. for intervals of zero, one, three, and six days prior to assay.

The method of Brown *et al. (3)* was used for plasma PBI determinations. A fusion (involving precipitation, washing, drying, and ashing of the plasma proteins) was performed on each of the two plasma subsamples and duplicate

Received for publication September 14, 1956.

[1] Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers University, the State University of New Jersey, Department of Dairy Industry, New Brunswick. This research was supported in part by Research and Marketing Funds (Sec. 10b) of the Agricultural Research Service, Dairy Husbandry Research Branch, U. S. Department of Agriculture.

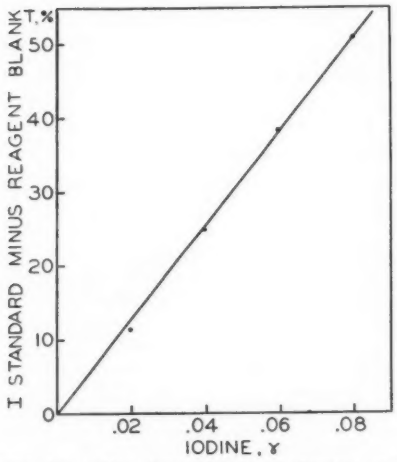

FIG. 1. Standard curve for iodine determinations adjusted such that the curve passes through the origin and demonstrates good linearity of response.

photometric determinations were made on each fusion sample, using a Klett-Summerson colorimeter. Klett-Summerson readings were converted to per cent transmittance by use of the following formula:

$$\% \text{ transmittance} = \text{Antilog } [2 - (\text{K-S reading} \times 0.002)]$$

A rectilinear relationship existed between iodine level and per cent transmittance. Figure 1 shows the manner in which a standard curve for iodine may be modified so that the curve passes through the origin. The reading of the reagent blank was subtracted from the reading of the iodine standard (or reading of unknown). A simple proportionality calculation was made from the following formula:

$$\text{PBI } (\mu\text{g. } \%) = \frac{\text{micrograms of iodine standard}}{(\text{reading of iodine standard minus reading of reagent blank})} \times (\text{reading of unknown minus reading of reagent blank}) \times 156.25 \text{ (dilution factor)}$$

A sample calculation follows:

Tubes	K-S reading	Transmittance	Mean transmittance
		(%)	(%)
Reagent blanks	420	14.45	14.45
	420	14.45	
Iodine standards (0.03 μg.)	243	32.66	32.89
	240	33.11	
Plasma unknowns	160	47.86	47.54
	163	47.21	

$$\text{PBI } (\mu\text{g. } \%) = \frac{0.03}{(32.89 - 14.45)} \times (47.54 - 14.45) \times 156.25 = 8.41$$

For each analysis series, reagent blanks and iodine standards in duplicate were carried through the entire procedure, and calculations of plasma PBI values were made according to the method illustrated.

The analysis of variance used in this study was according to the outline of Snedecor *(6)*. The expectations of mean squares for fixed and random effects were determined by the method of Schultz *(5)*.

RESULTS AND DISCUSSION

Mean plasma PBI values for this study are presented (Table 1), arranged by time and temperature of storage.

An analysis of variance (Table 2) of the plasma PBI data indicated the effects due to animal (or sample), animal × day, temperature, animal × temperature, and animal × day × temperature were significant ($P < 0.01$).

Components of mean squares and estimated variances were derived for the various sources of variation (Table 3). The variance for each source was also expressed as a per cent of total variance. Estimated variances may be further grouped under four main sources: animal; length and temperature of storage plus their interactions with each other and with animal; fusion; and determinations within fusion (Table 4).

TABLE 1

Effect of length and temperature of storage on plasma PBI

Day	Mean plasma PBI values		
	− 20° C.	2° C.	25° C.
	(μg. %)	*(μg. %)*	*(μg. %)*
0	5.73	5.08	5.04
1	5.39	4.96	5.00
3	5.55	5.93	5.07
6	5.00	5.32	4.78

TABLE 2

Analysis of variance of plasma PBI data

Source of variance	Degrees of freedom	Mean square	Error term	F
(Symbol)				
Total	191			
Animal — A	3	65.6555	F(ADT)	177.49[a]
Day — D	3	2.5626	AD	0.65
A × D — AD	9	3.9658	F(ADT)	10.72[a]
Temperature — T	2	2.9738	F(ADT)	8.04[a]
A × T — AT	6	1.7448	F(ADT)	4.72[a]
D × T — DT	6	0.6354	ADT	0.67
A × D × T — ADT	18	0.9529	F(ADT)	2.58[a]
Fusions in A × D × T F(ADT)	48	0.3699	C(F)(ADT)	7.67[a]
Determinations in fusions in A × D × T C(F)(ADT)	96	0.0483		

[a] Significant at the 1% level ($P < 0.01$).

TABLE 3

Components of mean squares and estimated variances

Source of variance	Mean square is an estimate of	Estimated variances	
(Symbol)		*(σ^2 or Θ^2)*	*(%)*
A	$\sigma^2C(F)(ADT) + 2\,\sigma^2F(ADT) + 48\,\sigma^2A$	1.36	65.0
D	$\sigma^2C(F)(ADT) + 2\,\sigma^2F(ADT) + 12\,\sigma^2AD + 48\,\Theta^2D$	−0.03	−1.4
AD	$\sigma^2C(F)(ADT) + 2\,\sigma^2F(ADT) + 12\,\sigma^2AD$	0.30	14.3
T	$\sigma^2C(F)(ADT) + 2\,\sigma^2F(ADT) + 64\,\Theta^2T$	0.04	2.0
AT	$\sigma^2C(F)(ADT) + 2\,\sigma^2F(ADT) + 16\,\sigma^2AT$	0.09	4.1
DT	$\sigma^2C(F)(ADT) + 2\,\sigma^2F(ADT) + 4\,\sigma^2ADT + 16\,\Theta^2DT$	−0.02	−1.0
ADT	$\sigma^2C(F)(ADT) + 2\,\sigma^2F(ADT) + 4\,\sigma^2ADT$	0.15	7.0
F(ADT)	$\sigma^2C(F)(ADT) + 2\,\sigma^2F(ADT)$	0.16	7.7
C(F)(ADT)	$\sigma^2C(F)(ADT)$	0.05	2.3
Total		2.10	100.0

TABLE 4

An evaluation of the main sources of variation

Combined sources of variance	Estimated variances	
	(σ^2 or Θ^2)	*(%)*
Animal	1.36	65.0
Handling effects[a]	0.52	25.0
Fusions in A × D × T	0.16	7.7
Determinations in fusions in A × D × T	0.05	2.3
Total	2.10	100.0

[a] Consists of day and temperature effects and their interactions with each other and with animal.

Direct effects of length and temperature of storage are seen (Tables 3 and 4) to contribute little to the total variance. However, the interactions of length and temperature of storage with each other and with animal (i. e., handling effects) contributed approximately 25% to the total variance, and is approximately 2.6 times the variance due to assay methodology (i. e., effects due to fusions and determinations within fusion). It is entirely unnecessary to have error variance due to these handling effects; they can be eliminated by the immediate recovery of plasma from the blood after collection, and processing the plasma without delay.

In this study, the variance attributable to variation among animals was 1.36 (or 65.0% of total variance). This may be considered low, because blood samples were used from cows in quite similar physiological states as expressed by age, and stage of lactation, thus tending to yield similar plasma PBI levels. Since only four cows were sampled, this estimate of animal variance cannot be considered very reliable.

Method error variance in this study was found to be 0.16 (fusion effects) plus 0.05 (determinations within fusion), for a total of 0.21. Thus, the standard error of a single plasma PBI determination was $\sqrt{0.16 + 0.05}$, or 0.46 μg. %. The coefficient of variation was 8.8%. Since approximately 77% of the method error variance was attributable to fusion effects, use of more fusion tubes in preference to more replicate determinations within fusion tubes would more effectively reduce error variance.

Use of two fusion tubes would reduce the standard error of a mean PBI determination to 0.32 μg. % and the coefficient of variation to 6.2%. Further increases in the number of fusion tubes would not reduce the error variance in a manner commensurate with the additional work required. It is recommended that in routine investigations, single photometric determinations be carried out on each of two fusion tubes.

SUMMARY

Effects of length and temperature of storage of plasma on the determination of plasma protein-bound iodine (PBI) by the alkaline fusion–ceric sulfate method were studied. Additional information was gained on the other method error term variances and on the derived standard error (S.E.) and coefficient of variation (C.V.), as a function of one or more fusions per plasma sample and one or more photometric determinations per fusion tube.

Direct effects due to length and temperature of storage of plasma contributed little to the total variance. Interactions of length and temperature of storage with each other and animal (i. e., handling effects) constituted 25% of the total variance and may be eliminated by processing and assaying plasma samples immediately after blood collection.

Based on method error term variance the standard error of a single plasma PBI determination was 0.46 μg. % and the coefficient of variation was 8.8%. The most effective method of reducing these values was to increase the number of fusion tubes per plasma sample.

ACKNOWLEDGMENT

The authors wish to acknowledge the counsel of R. E. Mather, Department of Dairy Industry, Rutgers University, regarding the statistical analysis of the data.

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EFFECT OF FEEDING CERTAIN SILAGES ON THE RELATIVE CONCENTRATIONS OF RUMEN VOLATILE FATTY ACIDS[1]

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Corn silage as the only roughage increased rumen acidity with more propionic and less butyric and acetic acids than did hay-crop silage used alone. Rumen acidity was not increased by either hay-crop or corn silage when they were part of the roughage ration. *Editor.*

In recent years considerable interest has been focused on the rumen fatty acids, their importance as a source of energy for the ruminant, and their possible implication in bovine ketosis. Since the various acids present have different energy values, information on factors affecting their relative concentration in the rumen is of considerable importance.

A number of investigators have shown that the relative concentrations of rumen volatile fatty acids may be affected by the diet. Tyznik and Allen *(8)*, for example, reported a relative increase in propionic acid production and a decrease in acetic acid production in cows on a restricted-roughage ration. Hibbs *et al. (6)*, working with calves, found differences in proportions of acids with different grain-to-roughage ratios. Card and Shultz *(3)* reported significant differences in the percentages of each of the three predominant acids due to general type of ration. Cutting date and methods of curing roughages were also found to have effects; later cutting increased acetic acid and decreased butyric, and silages fed as the sole roughage decreased acetic and increased propionic and butyric acids when compared with hay. El-Shazly *(5)* and Woodhouse *et al. (9)* have shown that high protein diets increase the butyric and higher fatty acids. Belasco *(1)*, using in vitro methods, reported that the total production and relative proportions of the volatile fatty acids are dependent on the type and amount of carbohydrate used.

It is very common practice to replace part of the hay in dairy cattle rations with silage. The study reported here was initiated to obtain information on the effect of feeding certain hay-crop and corn silages on the relative concentrations of the major rumen volatile fatty acids.

Received for publication August 30, 1956.

[1] Contribution No. 1063 of the University of Massachusetts, College of Agriculture Experiment Station, Amherst, Massachusetts.

EXPERIMENTAL PROCEDURE

Two mature Ayrshire cows, with permanent rumen fistulae fitted with plastic screw-cap cannulae, were used in this study. During Trials I and III both cows were in milk; during Trial II both were dry.

Two rations were compared in each trial, using a single reversal design with either two- or three-week adjustment periods, and 10- to 17-day sampling periods during which five to seven samples were taken.

The rations were fed twice daily in equal parts at 7 A. M. and between 3 and 4 P. M. The hay used in Trial I was second-cutting hay of good but somewhat variable quality; that in Trial II was first-cutting, year-old, field-cured hay, consisting largely of grasses. The corn silage fed in both Trials I and III was of excellent quality. Corn silage in Trial III, however, was more heavily eared. The-hay-crop silage used in Trial II was ensiled without preservative, in a gas-tight silo in late June; that used in Trial III was ensiled without preservative, in a gas-tight silo in mid-August. Both of these lots were light in legumes; the latter was somewhat weedy. To characterize better the roughages used in this study, average analyses of the various lots of silage and hay, as determined from routine periodic samples from each silo and composite samples from each lot of hay, respectively, are presented (Table 1). The concentrate used in all trials was a commercial 14-% fitting ration.

Rumen samples were collected at 11 A. M. (four hours after feeding), using a long curved metal tube fitted with a collection bottle and a bulb syringe for suction. This apparatus permitted collection from near the bottom of the rumen just ventral to the fistula. All rumen juice samples were collected from this general area of the rumen. Samples were taken immediately to the laboratory, and centrifuged at 2,000 r.p.m. for five minutes. A part of the supernatant liquid was used for pH determination, and the remainder was refrigerated for subsequent analysis (approximately one hour later).

Chromatographic separation of butyric, propionic, and acetic acids was accomplished essentially by the method for rumen juice developed by Linke *(7)* and outlined by Conrad *(1)*. This is an adaptation of the method of Buhlen *et al. (2)*. One of several modifications that were introduced was a slightly different solvent schedule, which, after careful standardization of reagents and procedure, permitted accurate separation of the acids without the necessity of collecting the eluant in small fractions. Butyric acid was not separated from the higher acids in the regular laboratory procedure, and this fraction was calculated as butyric acid and has been reported as butyric and higher acids. A limited number of separations made during the trials indicated that acids higher than butyric constituted approximately 1.3 to 3.1% of the total, on a molar basis. Before titration 50 ml. of CO_2-free distilled water and a few drops of detergent solution (Drene) were added to each beaker. Phenol red was used as an indicator. An electric stirrer was employed to insure thorough mixing of the two phases during titrations.

TABLE 1

Average composition of roughages[a]

Trial	Roughage	pH range	Acetic Acid	Butyric Acid	Lactic Acid	Moisture	Protein	Fat	Fiber	N. F. E.	Ash
						%					
I	Corn silage	3.70-4.15	6.70	None	6.28	78.4	11.1	2.2	34.3	46.7	5.7
	2nd-cutting hay	———	——	——	——	12.4	15.1	2.1	33.4	41.4	8.0
II	Hay-crop silage	4.50-5.40	3.29	4.28	None	74.7	10.9	3.5	43.9	34.2	7.4
	1st-cutting hay	———	——	——	——	8.5	6.2	1.8	35.4	51.9	4.7
III	Corn silage[b]	4.00	4.89	0.13	5.87	72.4	9.4	3.1	26.4	56.6	4.5
	Hay-crop silage	4.40-5.00	2.89	3.81	1.54	74.1	13.5	3.8	35.0	36.4	11.3

[a] All values except moisture and pH on dry-matter basis.
[b] One sample only.

TABLE 2

Rations, total volatile fatty acid levels, and relative percentages of fatty acids in rumen liquor

Trial		Ration (lb.)		No. Cows	No. Samples	Total V. F. A.	Relative percentage[a]		
							Butyric and higher[b]	Propionic	Acetic
						(mg/100 ml. rumen liquor)			
I	A	Corn silage	36	2	14	656.88	19.4	16.8	63.8
		2nd-cutting hay	12						
		Grain	5						
	B	2nd-cuting hay	24	2	14	613.95	19.7	16.9	63.4
		Grain	5						
II	A	Hay-crop silage	24	2	14	581.38	21.8	18.5	59.7
		1st-cutting hay	8						
		Grain	3						
	B	1st-cutting hay	16	2	14	586.12	19.0	19.2	61.8
		Grain	3						
III	A	Hay-crop silage	50	2	10	529.80	27.2	16.6	56.2
		Grain	10						
	B	Corn silage	50	2	10	619.02	24.9	21.4	53.7
		Grain	10						

[a] On a weight basis.

[b] Calculated as butyric acid.

All laboratory determinations were made in duplicate. Corrections were made for recovery of individual acids based on recovery of knowns during each trial. In all cases recoveries averaged 95% or higher. The total volatile fatty acid (V.F.A.) concentration was obtained by totaling the concentrations of "butyric and higher," propionic, and acetic acids.

The differences in fatty acid concentration between rations were tested for significance by analysis of variance, in which each sample was considered as an observation. It should be emphasized that this test does not permit broad inferences to be drawn regarding the effect of silages on rumen fatty acid concentrations, but it should be interpreted strictly in terms of the two cows used. A summary of the statistical data is presented (Table 3).

RESULTS AND DISCUSSION

The rations, total volatile fatty acid levels, and relative proportions of the acids for three trials are presented (Table 2). It will be noted that in Trial I corn silage, when fed to replace part of the second-cutting hay, slightly increased

TABLE 3

Analysis of variance of total volatile fatty acid levels and of relative percentages of fatty acids

Trial	Source of variation	d. f.	Mean squares[a]			
			Total V.F.A.	Butyric and higher	Propionic	Acetic
I	Treatment	1	12,901	0.32	0.01	2.41
	Cows	1	76,879[b]	0.17	1.24	2.64
	Interaction	1	7,517	9.43	0.45	6.61
	Error	24	3,794	2.37	1.09	1.94
II	Treatment	1	158	58.29	2.46	39.43
	Cows	1	126,680[c]	1.55	0.21	2.30
	Interaction	1	2,359	15.75[c]	5.79[c]	37.15[c]
	Error	24	3,267	1.24	0.55	1.25
III	Treatment	1	39,792[c]	25.09[b]	116.16[c]	38.92[b]
	Cows	1	10,597	25.54[b]	2.31	36.72[b]
	Interaction	1	1,692	1.06	0.34	4.14
	Error	16	2,701	2.45	0.51	3.19

[a] Where interaction was significant, it was used to test significance of treatment and cows; where interaction was not significant, error plus interaction was used.

[b] ($P < 0.05$).

[c] ($P < 0.01$).

total rumen fatty acid concentration. One cow showed a much greater response to silage feeding than the other. The difference in concentration between rations was not statistically significant. There was no appreciable effect on the relative proportions of the acids.

In Trial II, hay-crop silage, when fed to replace part of the first-cutting hay, had no appreciable effect on total rumen fatty acid concentration. The relative concentration of butyric and higher acids was increased, and that of propionic and acetic acids was decreased, when hay-crop silage was fed. Even though these differences were not statistically significant, because of a rather

large interaction mean square, they agree partially with the earlier work of Card and Shultz *(3)*, who obtained a relative increase in propionic as well as butyric acid concentrations when grass silage was fed as the sole roughage. The lack of complete agreement is perhaps not surprising, in view of a difference in level of grain and silage feeding, as well as the wide variations possible in the composition of hay-crop silages and hay.

Trial III was a direct comparison of corn silage versus hay-crop silage as the sole roughage. The corn silage ration resulted in a greater total rumen fatty acid concentration, a greater relative proportion of propionic acid, and smaller proportions of butyric and higher and acetic acids. All of these differences were found to be statistically significant.

Although the specific dietary factors responsible for the differences in fatty acid concentrations are not known at this time, the following observations are perhaps worthy of note: (1) Appreciable quantities of fatty acids were ingested in the silages, and there were appreciable differences in the acid composition between the corn and hay-crop silages. The rations which enhanced rumen butyric acid level were notably high in butyric acid. (2) In Trials II and III there was a difference in protein level between the two rations compared. This difference in both instances was in favor of the ration containing hay-crop silage. (3) In Trials II and III the rations containing hay-crop silage were lower in nitrogen-free extract and higher in crude fiber than those with which they were compared.

It may well be that a combination of these and possibly other factors is responsible for the differences observed.

SUMMARY

Two trials were conducted to study the effect on rumen volatile fatty acid concentrations of replacing part of the hay in the ration with corn or hay-crop silages. In a third trial, corn silage was compared with hay-crop silage as the sole roughage.

As part of the roughage, corn silage increased total rumen fatty acid concentration slightly but not significantly. The relative proportions of the acids were not altered. Hay-crop silage fed as part of the roughage did not enhance total rumen fatty acid concentration, but increased the relative proportion of butyric and higher acids and decreased that of propionic and acetic acids. These differences were not statistically significant.

Corn silage as the sole roughage resulted in a significantly higher total rumen fatty acid concentration, a significantly greater relative proportion of propionic acid, and significantly smaller relative proportions of butyric and higher and acetic acids than did hay-crop silage used as the only roughage.

ACKNOWLEDGMENT

The authors wish to express their appreciation to Drs. R. E. Smith and D. N. Stern of the Veterinary Science Department for their cooperation in fistulating the animals.

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INFLUENCE OF RATION AND RUMEN INOCULATION ON THE GROWTH OF DAIRY CALVES [1, 2]

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Effects of a high roughage ration and rumen inoculations on the growth and development of new-born calves were studied through four months of age. Restricting the grain intake decreased height at the withers, but did not affect roughage consumption, weight gain, or chest or barrel circumference. Rumen inoculations did not affect growth. *Editor.*

The dairyman is vitally interested in a calf-raising system which will give acceptable results and at the same time be economical and simple to follow. If the introduction of roughage into the calf's ration at an early age results in early development of the reticulo-rumen, this would conceivably effect substantial savings because it would be possible to raise herd replacements with a minimum amount of costly concentrates and liberal quantities of good forage. The evidence indicates that high roughage rations can be used advantageously in raising dairy calves *(5, 6, 7, 9, 10)* and that it is possible to encourage the utilization of forage at an early age by introducing into the calf rumen microorganisms obtained from mature, healthy animals *(3, 4, 17, 18, 19)*. Unfortunately, there is little information in the latter reports concerning the effect of rumen inoculations on growth rate in young calves. Several workers *(1, 11, 16)* have reported no increase in growth rate from rumen inoculations. McGilliard *et al. (13)* found that inoculations were helpful in preventing the decrease in weight gain in calves when aureomycin feeding was discontinued.

The objectives of this study were *(1)* to obtain additional information on the effect of diet on the growth of dairy calves and *(2)*, to study further the value of rumen inoculations under practical calf-raising conditions.

EXPERIMENTAL PROCEDURE

Thirty-two calves of the Holstein, Jersey, and Guernsey breeds were employed in this investigation. The calves were taken from their dams after they

Received for publication October 4, 1956.

[1] Contribution from the Department of Dairy Science.

[2] Data presented are based on a thesis submitted by G. A. Miller to the Graduate Faculty of the Virginia Polytechnic Institute, in partial fulfillment of the requirements for a Master of Science Degree.

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had received one feeding of colostrum, placed in individual stalls and hand-fed colostrum milk at the rate of 1 lb. per 10 lb. of live weight, divided into two feedings daily, for the first three days. Whole milk was fed at the same rate from four through 60 days of age. The calves were placed on the experiment at four days of age and allotted at random, within breeds, to the following treatments: Group A (control)—fed hay free-choice and a simple grain ration free-choice with a 4-lb. daily maximum; Group B—fed hay free-choice, but grain was restricted to one-half (by weight) of the amount of hay consumed; Group C—fed similarly to calves in Group A, except they were inoculated with fresh cud material at the approximate ages of five, ten, 15, and 21 days; Group D—fed similarly to calves in Group B, except that they were inoculated with fresh cud material at the approximate ages of five, ten, 15, and 21 days. The experimental treatments were imposed through four months of age. Care was taken to prevent cross-inoculation among the calves.

All of the calves were fed a grain ration composed of the following poundages: ground corn, 600; ground oats, 580; wheat bran, 480; soybean oil meal, 200; salt, 200, and steamed bonemeal, 20. Fair to good quality mixed hay was fed.

Feed consumption data were obtained on a daily basis for all calves. The calves were weighed and measured at the beginning and end of the experiment and at weekly intervals in-between. Blood glucose determinations *(15)* were made on all calves at two-week intervals throughout the experimental period.

RESULTS

A summary of the growth data is presented (Table 1). The mean daily body weight gain varied from 0.99 lb. for the calves in Group B to 1.21 lb. for the controls. The calves inoculated with fresh rumen material (Groups C and D) gained 1.07 lb. daily, compared with 1.10 lb. for the uninoculated calves. Calves restricted in grain consumption (Groups B and D) gained 1.02 lb. per head daily, compared with 1.16 for the calves allowed a daily maximum of 4 lb.

TABLE 1

Effect of ration and rumen inoculations on weight gains and certain body measurements

Group	Average daily gain[a]	Average increase in		
		Wither height[b]	Chest circum.[a]	Barrel circum.[a]
	(lb.)	*(in.)*	*(in.)*	*(in.)*
A—Hay free-choice, grain 4 lb. daily maximum	1.21	6.9	11.9	23.8
B—Hay free-choice, grain restricted	0.99	5.7	11.1	21.2
C—Hay free-choice, grain 4 lb. daily maximum plus inoculations	1.11	6.8	11.2	21.4
D—Hay free-choice, grain restricted plus inoculations	1.04	6.1	11.0	21.5

[a] None of these differences was statistically significant.

[b] The difference between the restricted and unrestricted grain-fed calves was significant at the 1% level.

of grain. None of the differences in weight gain due to rations or inoculations was statistically significant.

Restricting the amount of grain consumed resulted in a significant (P=0.01) decrease in the average increase in wither height, but had no essential effect on chest or barrel circumference. Rumen inoculations were observed to have no significant effect on increase in height at withers, or no increases in chest or barrel circumferences.

The feed data summarized (Table 2) indicate that in general the calves not restricted in grain feeding consumed approximately the same quantity of hay as the restricted calves (520 vs. 527 lb.). Calves limited in grain intake

TABLE 2

Effect of ration and rumen inoculations on feed consumption and efficiency of feed utilization

	Av. total consumption per calf				TDN per lb. gain[c]
	Milk	Grain	Hay	TDN[a,b]	
	(lb.)	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*
A—Hay free-choice, grain 4 lb. daily maximum	516.0	189.0	260.8	350.0	2.43
B—Hay free-choice, grain restricted	514.0	119.4	245.9	292.4	2.48
C—Hay free-choice, grain 4 lb. daily maximum plus inoculations	501.0	177.7	258.7	338.5	2.58
D—Hay free-choice, grain restricted plus inoculations	473.4	134.8	281.4	314.5	2.55

[a] Calculated from Morrison's *(14)* tables.

[b] The difference between the restricted and unrestricted grain-fed calves was significant at the 5% level.

[c] None of these differences was statistically significant.

consumed significantly less (P = 0.05) calculated TDN *(11)* than calves allowed up to 4 lb. of grain per day. The inoculated calves consumed an average of 2 lb. more grain and about 17 lb. more hay per calf than the animals not receiving rumen material. These differences were not significant. Analysis of variance revealed no significant differences in efficiency of feed utilization (calculated TDN per lb. weight gain) between treatment groups.

In Figure 1 are presented the mean changes in blood glucose levels. No treatment effects were observed. There was a sharp drop from the first to the third week followed by a levelling-off period, which extended from the third to the seventh week. Another sharp drop occurred from the seventh to the 11th week of age, after which the blood glucose appeared to stabilize around 50 to 55 mg. %.

DISCUSSION

Restricting the grain intake by feeding only one-half (by weight) as much grain as hay consumed depressed daily gains, increases in wither height, and increases in barrel and chest circumferences. All of these differences, except those associated with wither height, were found to be nonsignificant. The body weight data agree essentially with those of Holter *(11)*, who reported that

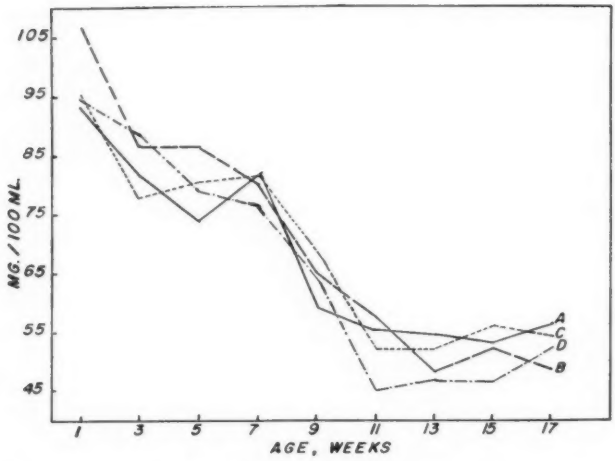

FIG. 1. Mean changes in blood glucose. (A = control, B = grain restricted, C = control ration plus inoculations, D = grain restricted plus inoculations.)

calves on high-roughage rations made lower body weight gains than control calves during a 16-week feeding period. Since no significant differences were noted in the pounds of TDN consumed per pound of gain (Table 2), it is indicated that the calves utilized the high-roughage ration as efficiently as the ration containing a larger proportion of grain.

The observation that the unrestricted grain-fed calves made significantly greater increases in height at withers than the restricted calves, but did not differ significantly with respect to weight gain, heart girth, or barrel circumference, is difficult to explain. Several possibilities exist. In view of the work by Conrad *et al. (3)* and Hibbs *et al. (8)*, the apparent greater skeletal development of the unrestricted calves may have been due to increased intakes of digestible energy and protein. However, there is no good reason to believe that such a condition would affect only skeletal development and not result in an increase of soft tissue. Another possibility is that since the restricted calves did not receive any grain until they were consuming at least one-half pound of hay per day, these animals may have suffered from an insufficient intake of phosphorus and/or a calcium-phosphorus imbalance during early life. Since some of the hay used in this study was of inferior quality, it appears reasonable to assume that it was deficient in phosphorus content. Thus, the major source of this mineral for the calves restricted in grain intake was the milk. The likelihood that one or more of the minor elements may have been involved can not be completely eliminated; however, since our knowledge of the animal's needs for these minerals and their specific function in the body is so meager, no definite statement can be made concerning them.

Results of this study show that rumen inoculations did not increase growth rate or efficiency of feed utilization in young calves. These are in general

agreement with the results of the Cornell workers *(11, 16)* and with the work of Ackerman and Fike *(1)*. On the other hand, these results may be considered to be at variance with the observations of the Ohio workers *(19)*, who reported that calves receiving fresh cud material from mature, healthy animals exhibited a better physical appearance than uninoculated calves.

It is reasonable to assume that if mature types of organisms could be established in the young calf by rumen inoculations, the calf could make better use of high-roughage rations and thus make more economical gains. Recently, Bryant and Small *(2)* have shown that inoculation of calves raised under practical conditions may hasten the development of rumen protozoa, but has little or no effect on the development of bacteria typical of the mature rumen. It appears that under practical management conditions the young calf becomes inoculated naturally at an early age by direct contact with older animals, through the ingestion of feed and water, through its bedding, and possibly through certain air-borne organisms.

The decline in blood glucose during the first ten to 11 weeks is interpreted as being due, in large part at least, to the effects associated with the functional development of the reticulo-rumen as suggested by McCandliss and Dye *(12)*. Thus, since no treatment effects were observed, it is indicated that neither the early consumption of roughage nor rumen inoculations had any effect on hastening this development.

SUMMARY

Thirty-two new-born dairy calves were used in studying the effects of a high-roughage ration and rumen inoculations on growth and development. The experimental treatments were imposed through four months of age.

The control calves gained an average of 1.21 lb. daily; those restricted in grain feeding, 0.99 lb.; those fed similar to the controls and inoculated, 1.11 lb., those restricted in grain feeding and inoculated, 1.04 lb. None of the differences between groups were statistically significant. Restricting the grain intake resulted in a significant ($P = 0.01$) decrease in the height at withers, but had no essential effect on chest or barrel circumference. Rumen inoculations had no significant effect on height at withers or on chest or barrel circumference.

No significant differences were observed in efficiency of feed utilization (calculated TDN per lb. body weight gain) between treatment groups; however, the calves limited in grain intake consumed significantly less ($P = 0.05$) TDN than calves allowed up to four pounds of grain daily. Restricting the grain intake did not materially affect the consumption of roughage. The inoculated calves did consume slightly more roughage than the uninoculated animals; however, the difference was not significant.

The development of the reticulo-rumen, as judged by the decline in blood glucose, was not affected by the amount of roughage in the diet or by rumen inoculations.

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EFFECTS OF COMBINATIONS OF FEEDSTUFFS, WITH AND WITHOUT AUREOMYCIN, ON IN VITRO DIGESTION OF CELLULOSE BY RUMEN MICROORGANISMS[1]

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The proportion of cereals with poor quality hay did not affect the amount of cellulose digested, and protein-rich feeds were interchangeable in protein-rich rations without affecting digestion. Aureomycin depressed cellulose digestion. *Editor.*

Effects of various nutrients and feedstuffs on the in vitro digestion of cellulose by rumen microorganisms have been investigated *(1-7, 10, 17-19)*. In most of these studies, only the effects of single feedstuffs on cellulose digestion were tested. Since it was established that the kind of feedstuff affected cellulose digestion, it seemed possible that different complementary effects might result with various combinations of feedstuffs. It seemed probable that such findings might be important in formulating practical rations and in interpreting feeding results that disagree. This research was initiated to study these problems in vitro with feed combinations used in dairy cattle feeding.

One of the investigations on various feedstuff combinations which seemed to be indicated was adding aureomycin in duplicated trials, because it has been shown to depress cellulose digestion in vitro *(11, 17, 18)*. Radisson *(17)* found that this inhibitory effect of aureomycin was considerably greater when filter paper was the source of cellulose than when alfalfa hay, bromegrass hay, or prairie hay was used. Radisson conjectured that aureomycin increased the requirement of rumen bacteria for a factor(s) not normally needed, or needed in much smaller quantities, for cellulolytic activity in the absence of aureomycin. Alfalfa hay, the ash of alfalfa hay, grain, urea, and soybean oil meal were found to be good sources of this factor(s), because these materials reduced or reversed the inhibitory effect of aureomycin on digestion of cellulose in vitro. This study was planned to ascertain the combinations of grains and protein-rich feeds used in practical feeding that would be most effective in overcoming the depressing effects of aureomycin on cellulose digestion. Also, it was hoped that aureomycin would accentuate any differences among rations by imposing a stress on microorganisms.

Received for publication September 28, 1956.

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EXPERIMENTAL PROCEDURE

The miniature artificial rumen and technique described by Huhtanen *et al. (9)* and modified by Radisson *(17)* were further modified for these experiments.

As used in this study, the miniature artificial rumen (Figure 1) consisted of dialysis tubing [3] 15/16-inch wide and 12.5 cm. long. This tubing was tied at one end with a string to form a sac into which the substrate and the rumen fluid, used as inoculum, were placed. This sac was then suspended in an 8-oz., wide-mouth jar containing approximately 100 ml. of McDougall's synthetic saliva

FIG. 1. Unassembled miniature artificial rumen—The glass insert is inserted into the dialysis sac and then into the No. 10 rubber stopper, so that the upper end of the sac extends about ¼-inch above the stopper. The No. 10 rubber stopper is inserted into the 8-oz. jar and the gas release mechanism inserted into the glass insert.

(12). The jar was inserted with a No. 10 rubber stopper having a hole 16 mm. in diameter, into which was inserted the open end of the fermentation sac. The sac was kept in place by inserting a glass tube about 3 cm. long and 16 mm. in diameter into the portion of the sac within the No. 10 rubber stopper. This glass

[3] Dialysis tubing No. 4465—A2, obtained from the Arthur H. Thomas Company, Philadelphia, Pa.

tube was fitted with a No. 1 rubber stopper in which was inserted a capillary tube 3 cm. long with a 1-mm. bore. A 2-inch length of rubber tubing with pinch clamp was attached to the capillary tube, to permit the manual release of gas at various intervals during the fermentation period. The No. 10 rubber stopper also was fitted with a 7-mm. capillary tube which served as an air escape, so as to equalize differences in air displacement which develop after the rubber stoppers are installed.

To emphasize any differences in digestibility which might result from additions of certain feedstuffs in various proportions, prairie hay of poor quality was used as the test roughage. Protein content of the rations was standardized to 18% crude protein [calculated from Morrison *(15)*, pp. 1086-1131] by adjusting the quantity of protein supplement incorporated into the mixtures. The proportion of 500 mg. of prairie hay to 200 mg. of concentrate mixture was used in the substrate, upon the assumption that an average dairy cow would be fed a somewhat similar proportion of hay and grain. Except for the first trial, the cellulose content of these feedstuff mixtures was standardized by additions of

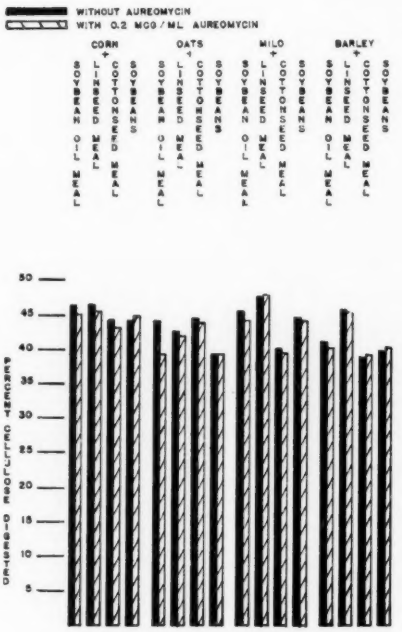

FIG. 2. In vitro microbiological digestion of cellulose in prairie hay and either corn, oats, milo, or barley, each in combination with each of soybean oil meal, linseed meal, and cottonseed meal—with and without aureomycin added to each combination.

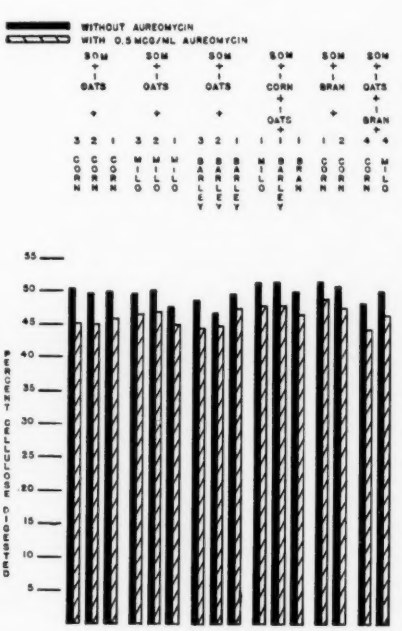
WITHOUT AUREOMYCIN
WITH 0.5 MCG./ML. AUREOMYCIN
SOM + 3 OATS + 2 CORN
SOM + 2 OATS + 1 CORN
SOM + 3 OATS + 1 MILO
SOM + 2 OATS + 1 MILO
SOM + 3 OATS + 1 BARLEY
SOM + 2 OATS + 1 BARLEY
SOM + 1 CORN + 1 OATS + 1 MILO
SOM + 1 CORN + 1 BARLEY
SOM + 1 BRAN + 2 CORN
SOM + 1 OATS + 1 BRAN + 4 CORN
SOM + 1 OATS + 1 BRAN + 4 MILO
DIGESTED CELLULOSE, MG.
55
50
45
40
35
30
25
20
15
10
5

FIG. 3. In vitro microbiological digestion of cellulose in prairie hay and certain combinations of soybean oil meal, oats, corn, and wheat bran with certain proportions of corn, milo, barley, wheat bran—with and without aureomycin added to each combination.

finely ground filter paper. The substrates consisting of prairie hay, grains, and filter paper were ground to pass through the fine screen (0.5-mm. mesh) of a Wiley mill.

After about 30 hours of incubation at 38° C., the contents of each artificial rumen were analyzed for cellulose *(8)*, and the amount digested was considered to be the difference between that which was found after incubation and that which was present in control samples of feed before incubation.

Four trials were conducted with the artificial rumen. Each of these trials included 64 artificial rumens which contained 16 different combinations of feedstuffs in duplicate without aureomycin, and the same combinations in duplicate with aureomycin. Each of the four trials was distinctive only in that it was not possible to run more than 64 artificial rumens simultaneously. The various combinations of feedstuffs tested in Trials I, II, III, and IV are shown (Figures 2, 3, 4, and 5).

When a single cereal grain was combined with a single protein-rich feed (Trial I, Figure 2), the proportion of one to another was determined by the

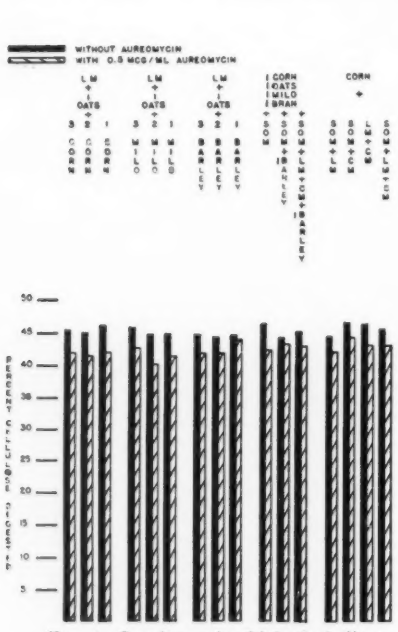

FIG. 4. In vitro microbiological digestion of cellulose in prairie hay and linseed meal plus oats, with certain proportions of corn, milo or barley; also in prairie hay and combinations of corn, oats, milo, wheat bran, soybean oil meal, linseed meal, and cottonseed meal—with and without aureomycin added to each combination.

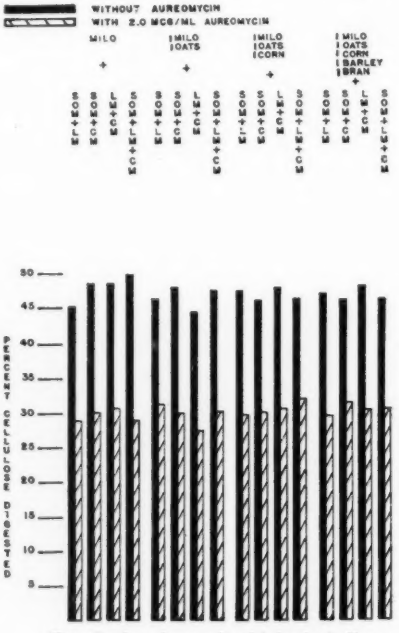

FIG. 5. In vitro microbiological digestion of cellulose in prairie hay and certain combinations of milo, oats, corn, barley, and wheat bran with combinations of soybean oil meal, linseed meal, and cottonseed meal—with and without aureomycin added to each combination.

weight of each required to make a ration containing 18% protein. The proportions (by weight) of each are shown (Figures 3, 4, and 5) for the concentrate rations that contained more than one cereal grain and by-product. Again, in each of these rations the proportion of protein-rich feed used was the quantity required to standardize each to 18% protein. When the concentrate ration included more than one protein-rich feed (Figures 4 and 5), the proportions of protein-rich feeds were equal by weight.

In Trial I, the quantity of aureomycin used was 0.2 γ per milliliter of rumen liquid. A similar concentration of aureomycin was placed in the synthetic saliva outside the sac. The concentration of aureomycin was increased to 0.5 γ per milliliter of rumen liquid in Trials II and III, and to 2.0 γ in Trial IV, to place even greater stress on the microorganisms.

RESULTS AND DISCUSSION

The mean difference between duplicates of 128 cellulose digestions was 1.2 percentage points with a standard error of $\pm$ .07. Thus, good reproducibility of the results obtained with the artificial rumen technique was demonstrated.

Effects of various combinations of feedstuffs on cellulose digestion (without aureomycin). The cereal grains, other than corn, were less constant in the rate of cellulose digestion than corn, occasionally being more efficient, but generally less efficient, than corn (Trial I, Figure 2). Therefore, nutrients required by rumen microorganisms to balance deficiencies of various protein-rich feeds appear to be found more uniformly in corn than in oats, milo, or barley.

Also, in the same trial linseed meal, when combined singly with each of the cereal grains, resulted in generally greater microbiological digestion of cellulose than any other single protein-rich supplement. Mixtures containing soybean oil meal ranked next in this respect.

Results in Trial I indicate that single cereal grains plus any of the protein-rich supplements affect the microbiological digestion of cellulose to different extents. This may be important in feeding certain combinations of feedstuffs and supplements.

Combinations of two or more cereal grains or by-products plus soybean oil meal exhibited less fluctuation, in per cent of cellulose digested (Trial II, Figure 3), than when single combinations of these feedstuffs were tested (Trial I, Figure 2). Therefore, higher numbers of feedstuffs in the combinations tested appear to supply a more adequate balance of nutrients required by the microorganisms which digest cellulose. Altering the proportion of any of the cereal grains or by-products had no consistent effect on the per cent of cellulose digested. This substantiates the equality of combinations of two or three cereal grains or by-products, with a protein-rich feed, in their influence on cellulose digestion.

Digestion of cellulose was uniform when combinations of linseed meal and certain cereal grains were tested (Trial III, Figure 4). Increasing the proportion of cereal grain in certain mixtures did not alter the per cent of cellulose digested. From the results of this trial, it is also indicated that the protein-rich feeds herein

tested can be interchanged in mixtures of protein-rich feeds without affecting the digestion of cellulose by rumen microorganisms in an artificial rumen.

The similarity in value found in Trial III (Figure 4) for the combinations of certain protein-rich feeds with various cereal grains tested was also found to apply to the mixture tested in Trial IV (Figure 5).

Burroughs *et al. (7)* and Ruf *et al. (19)* found that soybean oil meal or linseed meal stimulated in vitro cellulose digestion of a type of pure cellulose by rumen bacteria to a greater extent than cottonseed meal, when these protein-rich feeds were tested alone in the presence of some basic constituents of available nitrogen and minerals. In the study reported here, this was evident when these protein-rich feeds were incorporated into feed mixtures containing prairie hay and corn, milo, or barley (Figure 2).

The equally good milk production reported by Nevens *(16)*, which resulted from single protein supplements as compared with mixtures of them, corresponds to the results obtained here, when fairly similar combinations were compared for microbiological digestion of cellulose (Figures 4 and 5).

Simple grain mixtures containing one cereal grain and one protein-rich feed did not result in such consistently high percentages of cellulose digested (Figure 2) as those found with more complex mixtures (Figures 3, 4, and 5). This does not appear to correspond to the equality of simple grain mixtures, when compared with complex mixtures for milk production, as reported by Monroe and Krauss *(13, 14)*. This might suggest that the nutritive value of certain feedstuffs cannot be derived entirely by the microorganisms responsible for the digestion of cellulose.

Associative effects of the various grain mixtures appear to be lessened as the number of grains in the mixture are increased after two cereals are included. This absence of associative effect with mixtures of three or more grains supports the conclusions of Watson *et al. (20, 21, 22)* which are based on in vivo digestibility studies. Also, Ruf *et al. (19)* found no associative effect on in vitro cellulose digestion when the effects of single feedstuffs added to wood cellulose, urea, and minerals were compared with combinations of two of these feedstuffs.

Effects of various combinations of feedstuffs on cellulose digestion in the presence of aureomycin. The addition of 0.2 γ aureomycin per milliliter of rumen liquid generally depressed the digestion of cellulose with most of the combinations of feedstuffs tested (Trial I, Figure 2), as compared with the same feedstuffs without aureomycin. There were a few increases in cellulose digestion resulting from the addition of aureomycin at this level, in mixtures containing raw soybeans and either corn, oats, or barley, and in the mixtures containing linseed meal and milo, or cottonseed meal and barley. However, these differences were so small that they could be of only minor significance.

The increased quantities of aureomycin added in Trials II, III, and IV (Figures 3, 4, and 5) caused a comparatively uniform reduction of the per cent of cellulose digested in the presence of each of the combinations or proportions of ingredients tested. Therefore, the combinations and proportions of feedstuffs

tested in these trials might be assumed to be of equal value for microbiological digestion of cellulose under the conditions of stress imposed by the aureomycin.

SUMMARY AND CONCLUSIONS

Combinations and proportions of cereal grains and protein-rich feeds (all standardized to 18% protein) with prairie hay were tested for their effect on the in vitro rumen microbiological digestion of cellulose, both with and without aureomycin added.

Simple mixtures of single cereal grains with single protein-rich feeds added to prairie hay were found to differ in percentage of cellulose digested. Mixtures containing corn in combination with several single protein-rich feeds fluctuated less in percentage of cellulose digested than any other mixture of a single cereal with a single protein-rich supplement. When mixtures of certain feedstuffs containing three ingredients were used, the percentage of cellulose digested was as high as for mixtures containing a greater number of feedstuffs.

Certain feedstuffs included in mixtures of three or more ingredients appear to be interchangeable, as measured by their effects on the per cent of cellulose digested. Altering the proportion of certain cereal grains in the mixtures tested did not affect the percentage of cellulose digested.

Apparently, protein-rich feeds can be interchanged in mixtures of protein-rich feeds without affecting the digestion of cellulose by rumen microorganisms in an artificial rumen.

Aureomycin, with the respective levels used, was found to depress cellulose digestion to a similar extent with all but a few of the mixtures tested. As the concentration of the antibiotic was increased, digestion of cellulose decreased with all of the mixtures tested. With a few feed combinations, a low level of aureomycin increased slightly the digestibility of cellulose, but differences were so small that they could be of only minor significance.

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PROTEIN PRODUCTION IN THE BOVINE. DAILY PRODUCTION OF THE SPECIFIC MILK PROTEINS DURING THE LACTATION PERIOD[1]

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The synthesis of α-casein, β-casein, α-lactalbumin, and β-lactoglobulin was mutually related and reached a maximum level about five days after parturition. The daily levels of these proteins were unrelated to those of γ-casein, the immune globulins, and milk serum albumin. Maximum protein synthesis occurred about five days after parturition; that of fat, lactose, and total milk volume required about one month. *Editor.*

In recent years, the major specific proteins of bovine skimmilk have been isolated and characterized. Casein is composed of three components designated as α-casein, β-casein, and γ-casein. Five major components, the immune globulins (immune euglobulins and pseudoglobulins), α-lactalbumin, β-lactoglobulin, and milk serum albumin, together comprise up to 85% of the serum (or whey) proteins. These major proteins account for about 97% of the total protein of skimmilk *(3)*.

The biological and technological importance of each of these proteins have prompted an investigation of their production by the dairy cow. In this connection, studies reported from this laboratory have shown that there are differences between the various breeds of dairy cows in their relative production of the individual proteins *(10)*. The daily production of total casein and the milk serum proteins also has been compared with that of fat and total milk during the entire lactation period *(7)*. The studies reported herein describe the daily production levels of the individual proteins comprising casein and the serum proteins during the entire lactation period.

METHODS AND PROCEDURES

In order to clarify some of the current confusion in terminology, a review and comparison of the older and newer nomenclatures of the milk proteins have been made recently *(3)*. It should be noted that β-lactoglobulin is the major constituent and α-lactalbumin and milk serum albumin are the smaller constituents of the classical "lactalbumin" fraction. The immune globulins compose the majority of the classical "lactoglobulin" fraction. Typical electrophoretic patterns of total casein and of the total milk serum proteins are

Received for publication October 25, 1956.

[1] Supported in part by aid from the Rockefeller Foundation.

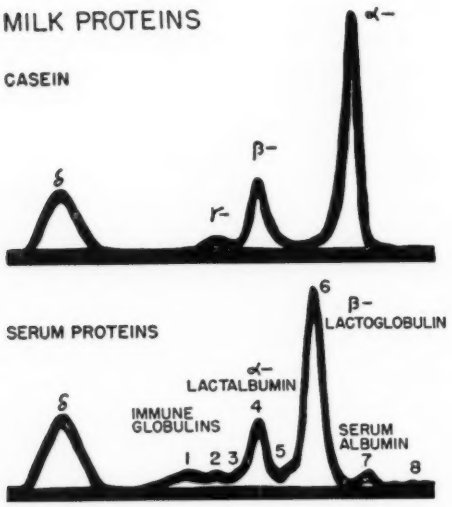

FIG. 1. Electrophoretic patterns of the milk proteins. (Sodium veronal buffer, pH 8.6, 0.12 M, ionic strength of 0.1, field strength of 7.8 volts per square centimeter, 1.2° C., 5,580 seconds. Protein concentrations were about 1.5 and 2.3%, respectively, for the casein and the serum protein solutions.)

shown (Figure 1). The three casein components and the five major serum protein components are evident. Components 1 and 2 of the serum proteins together constitute the immune globulins (euglobulins and pseudoglobulins, respectively); whereas, the other major components (α-lactalbumin, 4; β-lactoglobulin, 6; and milk serum albumin, 7) are single peaks. Components 3, 5 and 8 constitute proteins as yet unidentified, or part of the classical "proteose-peptone" fraction. Also, some proteins probably are present with the same mobility as the four major components, but are obscured because of their low concentration.

Since the intricacies of the electrophoretic analysis procedure limit the number of samples that can be analyzed, an intensive study was made with a few animals instead of making an incomplete study on many animals.

Background data for the eight cows studied are shown (Table 1). The production of the individual casein proteins by two of these animals (C and D) and the individual serum proteins by three of the animals (B, C, and D) will be considered through one lactation period of about 330 days. Cow A aborted at 267 days and was dropped from this experiment at that time. Cows E through H were studied during the first three months of their lactation periods, to establish more precisely the production of the individual casein and serum proteins at the beginning of the lactation period.

The calves were confined immediately after birth and were not permitted to nurse. The colostrum or first milk was removed by hand-milking, weighed, and an aliquot taken for analysis. Samples were taken from each milking for

TABLE 1
Parturition history for the experimental animals

Cow No.	Breed	Month of parturition	Age at parturition	Previous calves
			(mo.)	*(No.)*
A	Holstein	November	26	0
B	Guernsey	December	89	5
C	Guernsey	January	27	0
D	Holstein	February	83	4
E	Ayrshire	July	40	1
F	Holstein	August	40	1
G	Guernsey	August	46	1
H	Holstein	September	70	3

the first week of lactation, and then at less frequent intervals during the remainder of the lactation period. The morning and evening milks for each day were warmed to 37° C. and before analyzing were composited according to weight.

Solutions of casein and the serum proteins were prepared for the electrophoretic analyses from skimmilk according to previous procedures *(5, 10)*. These involve two precipitations of the casein at pH 4.65 with washing and dialysis and concentration of the serum protein solution. It was found necessary to dilute the first few samples after parturition with one or two volumes of distilled water, in order to precipitate the casein completely.

Total protein, casein, serum protein, and nonprotein nitrogen were evaluated on all of the milk samples according to the procedures of Rowland *(11)*. Recovery of the protein in the actual electrophoretic solutions was equivalent to 93 to 97% of the total nitrogen present in each of the Rowland casein and serum protein fractions. Thus, the protein present in the electrophoretic solutions represented effectively the protein present in the original skimmilk *(5)*.

Electrophoretic analyses in this investigation were done as previously described *(4, 5)* with the same conditions as specified in Figure 1. The total area of the electrophoretic patterns for the serum proteins was divided into four areas representing the four major components. Thus, in addition to the usual electrophoretic anomalies, the absolute amount and the percentage composition ascribed to these components may be too large. For example, minor components with the same or near the same mobility as β-lactoglobulin are also included in its total *(5)*. In addition, components are present in the first milkings after parturition which do not occur normally in milk or do so in small quantities. Thus, on the day of parturition, correction has been made for an unknown component with a mobility close to that of β-lactoglobulin. In a similar manner, correction has been made also for a component of casein with a mobility between that of α- and β-casein. This latter component has been noticed also in normal casein of mid-lactation, where its concentration is usually less than 1% of the total casein.

Data (Table 2) show the effect of the concentration of protein in the electrophoretic solutions on the relative area ascribed to each component. The apparent

TABLE 2

Effect of protein concentration used in the electrophoretic analysis on relative area ascribed to the various components

Protein component	Protein concentration (g/100 ml.)[a]					
	0.5	1.0	1.5	2.0	2.5	3.0
	(% of area due to each component)					
Casein:						
α-Casein	59.7	.....	65.8	66.3	64.0	.....
β-Casein	30.9	.....	28.4	28.6	28.4	.....
γ-Casein	11.9	.....	5.6	5.3	5.1	.....
Serum proteins:						
Immune globulins	.....	13.5	10.5	9.9	9.4	9.1
α-Lactalbumin	.....	18.8	21.0	19.0	18.3	18.2
β-Lactoglobulin	.....	62.9	65.0	67.3	69.2	70.7
Milk serum albumin	.....	4.7	3.5	3.8	3.1	2.0

[a] These concentrations represent the levels at which a standard solution of casein and the serum proteins were diluted with veronal buffer and analyzed in the electrophoretic apparatus, under the conditions specified in Figure 1.

concentration of γ-casein in the dilute solution and of β-lactoglobulin in the concentrated solutions appear anomalous. Thus, for uniformity all of the samples were analyzed at similar concentrations to make direct comparison possible, and the data reported in this paper are for casein solutions of 1.5 to 2.0% and for serum protein solutions of 2.0 to 2.5%.

RESULTS

The curves in Figures 3 and 6 show that the maximum daily production of α- and β-casein was reached about five days after parturition. The total production of α-casein was larger for some of the cows on the day of parturition; however, the decreased production on the first or second day, with the increase to the maximum in a few days, suggests that the large yield on the day of parturition was owing to that which had been stored in the mammary gland during the dry period. The daily production of α- and β-casein, after reaching the maximum, then declined to the end of the lactation period, with a slight increase shown at the time of maximum milk yield. In contrast to this, the daily milk yield kept increasing to a maximum after a month and then started decreasing until the end of the lactation period (Figures 2 and 5).

The daily production of γ-casein did not follow that of α- and β-casein. It was more variable and high in the colostrum of all of the animals the first day after parturition. The daily production of γ-casein by the Holstein cows remained high during the lactation period and did not decrease with the milk yield as much as α- and β-casein did. This caused a change during lactation in the γ-casein content of the total casein of the Holstein cows, as is shown for one cow (Table 3). For comparative purposes, the casein composition for cow C (a Guernsey) which did not change much is also shown. Studies on breed and individual variations in the specific protein constituents of milk have shown that the mid-lactation milk of Holstein cows contains significantly more

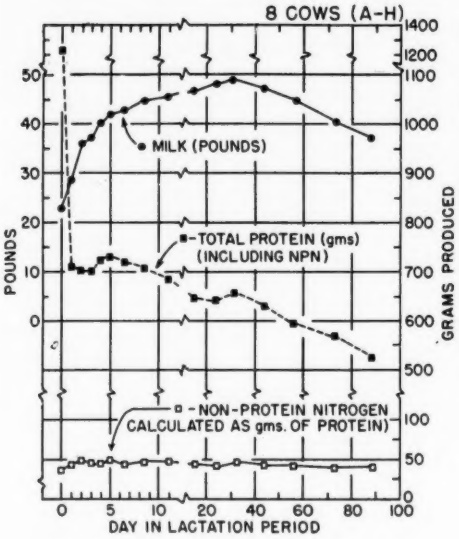

FIG. 2. Daily milk, total protein, and nonprotein nitrogen yields during the first third of the lactation periods.

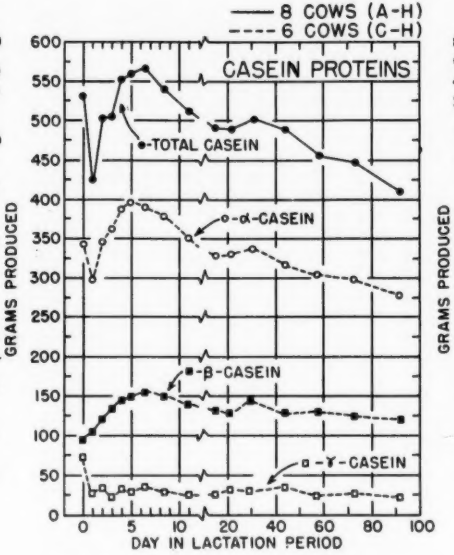

FIG. 3. Production of the specific casein proteins during the first third of the lactation periods.

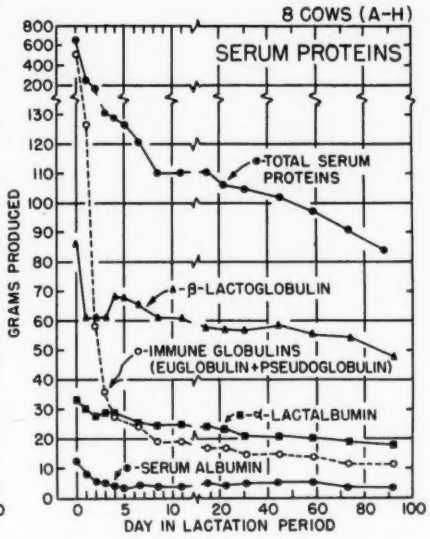

FIG. 4. Production of the specific milk serum proteins during the first third of the lactation periods.

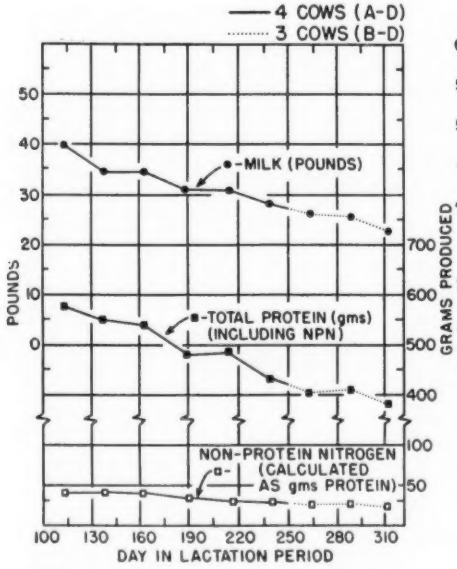

FIG. 5. Daily milk, total protein, and nonprotein nitrogen yields during the last two-thirds of the lactation periods.

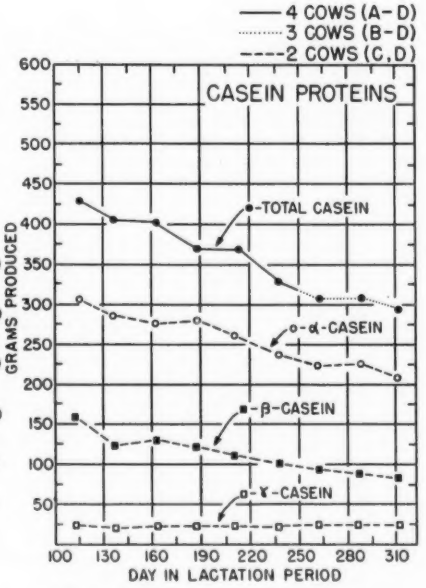

FIG. 6. Production of the specific casein proteins during the last two-thirds of the lactation periods.

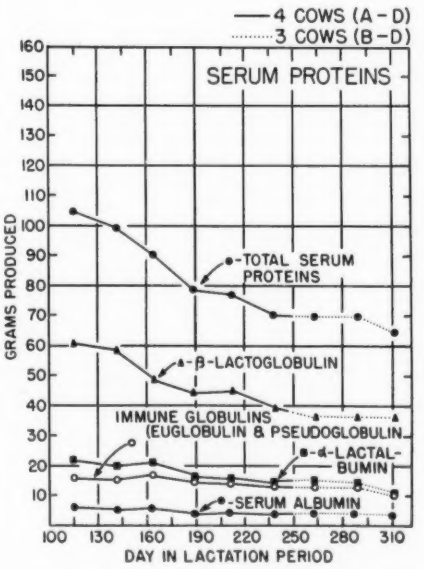

FIG. 7. Production of the specific milk serum proteins during the last two-thirds of the lactation periods.

TABLE 3

Changes in the composition of the specific proteins of casein and the serum proteins during the lactation period

Cow No.	Casein or serum protein component	Approximate day in lactation period [a]					
		0	30	100	200	300	330
		(% of total casein or serum proteins)					
C (Guernsey)	α-casein	73	65	67	67	69	70
	β-casein	19	30	30	30	27	27
	γ-casein	8	5	3	3	4	3
	immune globulins	70	11	12	13	15	19
	α-lactalbumin	21	21	20	19	17	15
	β-lactoglobulin	6	64	63	63	64	62
	serum albumin	3	4	5	5	4	4
D (Holstein)	α-casein	78	68	67	67	66	67
	β-casein	14	27	27	26	26	20
	γ-casein	8	5	6	7	8	13
	immune globulins	82	15	15	18	18	25
	α-lactalbumin	4	26	23	21	20	19
	β-lactoglobulin	12	53	55	55	55	50
	serum albumin	2	6	7	6	7	6

[a] With the exception of the first day, all figures are averaged values of two or three composited daily samples, taken on days on or near the designated day.

γ-casein than the milk of the other four major dairy breeds *(10)*. These differences in γ-casein production are apparently a reflection of a breed difference.

Data (Table 3) show that the percentage of α-casein in the total casein remained quite constant during the lactation period after the first few days. This has been noted previously by McMeekin *(9)*. The percentage of β-casein in the total casein was lower in the first milkings, increased, and then for the Holstein cows decreased, as the percentage of γ-casein increased. The relatively lower amount of β-casein in the first milking or two apparently reflects the fact that β-casein is absent or present in small amounts; whereas, there are always α- and γ-casein present in the mammary glands of dry cows *(8, 9)*. Thus, the high yield of total casein in the first milkings of some cows probably was caused by the accumulation of α- and γ-casein in the mammary gland during the dry period.

Production curves for the specific serum proteins are shown (Figures 4 and 7). The daily production of α-lactalbumin and β-lactoglobulin follows closely that of α- and β-casein and suggests that a maximum in daily production also was reached in about five days after parturition; although, like α-casein, some of the animals had a higher total production on the day of parturition. The β-lactoglobulin content of the serum proteins was relatively constant after the first few days. The Holstein cows had levels of around 50 to 55% β-lactoglobulin; whereas, the Ayrshire and the Guernsey cows maintained levels of around 60 to 65%. The lower amount is apparently a characteristic of the Holstein breed *(10)*. The α-lactalbumin composition of the serum proteins tended to reach a maximum about a month after calving and then decrease a little as the lactation period progressed.

Production curves for the immune globulins indicate that the total production was highest on the day of parturition, due mainly to an accumulation during the dry period *(8)*, and that it dropped to the end of the lactation period. Near the end of the lactation period, the daily production of the immune globulins did not drop as rapidly as that of the other proteins. Since the daily milk yield started decreasing after the first month or so, the relative concentration of the immune globulins tended to increase in the milk and in the total serum proteins, even though no increase in the total amount secreted daily took place (see Table 3).

Production curves for milk serum albumin reveal that, like γ-casein and the immune globulins, the daily production fluctuated somewhat during the lactation period and in all cases the amount of milk serum albumin was highest in the milk on the day of parturition. On a protein percentage basis, however, the level present was about the same as in normal milk (about 1.0% of the total protein). The increased amount of serum albumin on the day of parturition was probably that which had accumulated during the dry period *(9)*. It should be noted that the cows tended to secrete more serum albumin, immune globulins, and γ-casein in the colostrum than the first-calf heifers did.

DISCUSSION

Production curves suggest that there is a close similarity in the daily production of α-casein, β-casein, α-lactalbumin, and β-lactoglobulin during the lactation period. Previous work has indicated that the amounts of these four proteins present in the mid-lactation milk of individual cows are highly correlated; whereas, there is little relationship between the amounts of these proteins and γ-casein, the immune globulins, or milk serum albumin *(10)*. These observations suggest that α-casein, β-casein, α-lactalbumin, and β-lactoglobulin are proteins synthesized in close relationship to each other in the mammary gland. The more variability in the production of γ-casein, the immune globulins, and milk serum albumin suggests that the amounts of these proteins present in the milk are dependent upon other factors.

Askonas *et al. (1, 2)*, by the use of radioactive tracers in goats and rabbits, have shown that total casein (containing only a trace of γ-casein) and β-lactoglobulin are synthesized in the mammary gland from the free amino acid pool; whereas, the immune globulins enter the gland in a bound or preformed state from the blood. This suggests by analogy between the species that α-casein, β-casein, and β-lactoglobulin are synthesized in the mammary gland, whereas the immune globulins are not. Since milk serum albumin is identical to the serum albumin of blood, it is probable that it also enters the mammary gland in a preformed state from the blood. Studies are now in progress in our laboratory to determine with C^{14} tracers which of the major milk proteins of the bovine are synthesized in the mammary gland and which enter the gland in a preformed or bound state from the blood.

It is apparent from data of Figures 2, 3, and 4 why previous studies on the protein composition of milk which have ignored the first part of the lactation period, or have used individual or composited weekly or monthly samples, have failed to appreciate how early in the lactation maximum protein synthesis is attained in the mammary gland *(7)*. It is well known that the dairy cow tends to reach a maximum of production for total milk and fat about a month after parturition. Cows used in this study were normal in that respect. The low lactose content of colostrum and milk at the start of lactation, and the rather constant composition during the remainder of the lactation period, suggest that the same is also true for lactose. In contrast, these studies suggest that the cow is in full total protein production at the start of the lactation period, owing to proteins accumulated during the dry period, and actually reaches full synthetic production about five days after parturition. These observations have been summarized (Table 4), with the distinction being made between the days of maximum total production and of apparent maximum daily synthesis. The ability of the cow to reach a maximum synthesis of milk

TABLE 4

Comparison of the days of maximum production and of maximum mammary gland synthesis of the milk constituents

Total milk or constituent of	Approximate day in lactation of maximum production	Approximate day in lactation of maximum synthesis
Milk	30	30
Fat	30	30
Lactose	30	30
Protein (total):	0	5
α-casein	5	5
β-casein	5	5
γ-casein	0	?
immune globulins	0	?
α-lactalbumin	5	5
β-lactoglobulin	5	5
serum albumin	0	?
nonprotein nitrogen	5	—

proteins and at the same time to start replacing the immune globulins lost in the colostrum back into the blood serum *(6)*, exemplifies the large amino acid requirements of the cow in the first few days of the lactation period.

SUMMARY

Recent advances in the knowledge of the milk proteins prompted a study of the daily production levels of the individual proteins during the complete lactation period.

The daily production levels of α-casein, β-casein, α-lactalbumin, and β-lactoglobulin tended to be closely related to each other and attained a maximum about five days after parturition. Little relationship existed between the daily

production levels of these four proteins and those of γ-casein, the immune globulins, and milk serum albumin.

These studies suggest that maximum protein synthesis is attained in the bovine mammary gland about five days after parturition, in contrast to the synthesis of fat, lactose, and total milk volume, which reaches a maximum about a month after parturition.

ACKNOWLEDGMENTS

The authors wish to acknowledge the laboratory assistance of G. D. Rolleri, G. W. Sonnenfeld, and W. T. Cunningham, and the help of the farm foreman, R. L. Shirley, in this work.

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EFFECT OF SEASON ON SECRETION OF IODINE IN MILK[1, 2, 3]

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A significant seasonal effect on the metabolism of iodine by the dairy cow was shown with I^{131} in single and in continuous daily doses. In the spring, a stimulant appeared which increased the blood and milk levels of this element and the mammary gland concentrated it in the milk. *Editor.*

A marked seasonal variation in the iodine content of thyroids of beef cattle was reported by Seiden and Fenger *(9)* in 1913. Similar variations have been noted in thyroids of sheep and swine. In cattle, the lowest concentration was found during January and February. After these months the thyroids exhibited so marked an avidity for iodine that during June to November they contained three times as much of the element as during December to May. In sheep *(3)*, the secretion of thyroxine was at a maximum in March and was lowest in July, thus sugesting that the iodine content of the thyroid gland may be inversely related to secretory activity.

In dairy cattle, the concentration of iodine in milk shows a marked seasonal variation *(6)*, with a low being found during the late spring and a high during August. Other workers *(2, 8)* have agreed as to the marked variations in the iodine content of milk, but found the low iodine levels to occur from April to September and the peak concentrations from October to March. These differences among results of workers are hard to reconcile. The possibility of variations in dietary iodine in the different seasons should be considered.

In the experiments reported here, the effect of stage of lactation, and level of production, also the effect of season of the year on the amount of I^{131} appearing in the milk and on the ability of the mammary gland to concentrate iodine in milk, have been noted.

Received for publication November 7, 1956.

[1] This manuscript is published with the permission of the Director of the University of Tennessee Agricultural Experiment Station, Knoxville, Tenn.

[2] The radioactive materials used in this work were obtained from the Oak Ridge National Laboratory on allocation from the United States Atomic Energy Commission.

[3] This work was completed under Contract No. AT-40-1-GEN-242 between the University of Tennessee, College of Agriculture, and the Atomic Energy Commission.

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EXPERIMENTAL METHODS

Two groups of grade Jersey cows were used. One group was fed a low-iodine ration, to study the effect of lactation, pregnancy, and season on the uptake of I^{131} by the thyroid. These cows received each day about eight pounds of a 16-% protein dairy ration and all of the mixed hay they would consume. The daily iodine intake was approximately 3.5 mg. They were kept in box stalls and were allowed free access to water and noniodized salt. At the start of the test period each cow was given 100 μc of I^{131} by intravenous catheterization. The milk was then collected for a period of one week and the radioactivity of the thyroids determined for two weeks.

The second group of cows was kept in a separate shed and fed from four to 12 lb. each of a commercial dairy feed (18% protein) and all the mixed hay they would consume. They were kept on this regime during their entire lactation. The iodine intake varied from 12 to 16 mg. per day. During the experimental period these cows were placed in metabolism stalls and dosed with five millicuries of I^{131} either intravenously or orally. A feed balance and the collection of milk, blood, urine, and feces were carried out during the seven-day period after dosing.

For determination of the I^{131} content of the samples, an accurately measured amount of each of the samples (from 0.5 of a ml. to 3 ml.) was placed in a 16×150 mm. test tube and counted in a deep-well scintillation counter that employed a thallium-activated sodium iodide crystal. External thyroid counts were made using a scintillation probe designed by Monroe (*7*), that contained a thallium-activated sodium iodide crystal and was connected to a count-rate meter.

RESULTS

The concentration of radioiodine in the milk of three cows at three stages of the lactation period is presented (Table 1). From these results it appears that there is no consistent influence of stage of lactation per se, on either the total amount of iodine secreted or the concentration of iodine. The observations are confounded by season, since even the shortest lactation was six months long. It should be noted that the lactations did not all start at the same season of the year.

TABLE 1

I^{131} content of milk of cows dosed several times during course of lactation

Stage of lactation	Total % in milk in 7 days			Per cent of dose $\times 10^{-4}$ per ml. of milk 24 hr. after dosing		
Cow	A	H	C	A	H	C
First third	16.36[a]	4.51[b]	4.65[c]	3.82	1.66	2.78
Middle third	5.96	4.14	7.94	1.87	2.48	5.46
Last third	10.78	6.20	3.85	7.09	3.82	4.11

[a] Normal iodine ration; lactation started May 31, 1954.
[b] Low iodine ration; lactation started December 23, 1954.
[c] Low iodine ration; lactation started January 2, 1955.

TABLE 2

Effect of level of production and season of year upon the seven-day secretion of I^{131} into milk

Daily milk yield	January to March		April to July	
	Observations	Av. amount I^{131} in milk	Observations	Av. amount I^{131} in milk
(lb.)	*(No.)*	*(%)*	*(No.)*	*(%)*
Less than 15	5	5.17	5	5.54
15 to 25	8	5.62	8	7.01
25 to 35	1	4.51	2	13.17
35 and over	0	...	1	7.53

A summary is presented (Table 2) of 30 observations made on 11 different cows over a period of nine months. The level of production did not have a striking effect on the total per cent of I^{131} secreted in milk. Dividing the data into periods of collection revealed that at all levels of production more iodine was secreted in the milk during the months of April to July. In the five cases where comparisons were possible, it was noted in every instance that the effect of the advance of spring was to cause more of the I^{131} to be secreted in the milk. The average concentration of I^{131} in milk at 24 hours after dosing increased from 0.29- to 0.48-% of the dose per kg. Since two of the cows produced at nearly the same levels during these periods, a decrease in the level of production may be disregarded as a significant factor in the differences.

The data for the concentration of I^{131} in milk and in the thyroids of the low-iodine cows are summarized (Table 3). These indicate that in all cows there was a rise in the concentration of I^{131} in the milk, from a low value during January to a higher concentration in May, with this effect becoming apparent as early as March. A semi-logarithmic plot of the data for each cow showed no change in the rate of decline of the I^{131} content of milk. The I^{131} concentration decreased at the rate of 50 % every half-day.

Thyroid counts were made coincidental with the milk collections. The values represent the maximum uptake exhibited. Three of the five animals had an increase in the I^{131} level in the thyroid from January to March. All cows exhibited a marked drop in the thyroid I^{131} during May. A graphical plot of the thyroid iodine uptake and release curves during this test indicated that the low thyroid levels in May were due to a failure to accumulate the I^{131}, rather than to an increased turnover rate.

TABLE 3

I^{131} concentration of milk collected five hours after intravenous injection of the radioactivity, and maximum thyroid accumulation of I^{131} of cows on a low-iodine ration

Cow	1-3-55		3-21-55		5-2-55		6-13-55
	Milk	Thyroid	Milk	Thyroid	Milk	Thyroid	Thyroid
	(%dose/kg)	*(% dose)*	*(%dose/kg)*	*(% dose)*	*(%dose/kg)*	*(% dose)*	*(% dose)*
G	.....	17.2	0.73	15.0	0.85	8.2	13.1
H	0.43	15.9	0.77	20.0	0.78	6.1	.....
C	0.32	29.9	0.44	21.0	0.66	11.7	.....
I	0.43	16.6	0.46	22.1	0.55	9.9	15.1
E	0.78	19.4	1.06	24.6	1.11	.....	.....

TABLE 4

Effect of seasonal influence upon secretion of iodine in milk[a]

Cow	Date of trial	Daily milk yield	Total I^{131} in milk in 7 days	24 hour conc.		I^{131} conc. of milk
				Milk	Blood	I^{131} conc. of blood
		(lb.)	*(% of dose)*	*(% dose × 10^{-4}/ml)*		
A	1-5-55	20	6.0	2.42	2.49	0.97
B	1-17-55	11	5.8	4.17	3.19	1.31
A	1-27-55	20	6.8	2.54	2.31	1.10
B	2-10-55	11	6.6	3.88	3.52	1.10
B	3-2-55	11	5.4	3.44	3.66	0.94
C	3-7-55	18	4.1	2.48	2.30	1.08
D	3-10-55	15	6.6	3.66	2.77	1.32
E	4-8-55	15	6.0	2.76	1.42	1.94
A	4-20-55	13	10.8	6.86	3.23	2.12
E	4-28-55	15	6.8	3.20	1.42	2.25
F	5-11-55	44	7.5	1.95	1.35	1.44
C	5-16-55	10	6.2	3.80	1.64	2.31
A	5-20-55	8	12.3	8.73	1.57	5.55
A	7-27-54	30	16.4	3.82	.....	.....
A	10-25-54	24	6.5	2.28	.....	.....

[a] All values for milk I^{131} have been corrected for time of secretion by plotting the experimental values as the values for the median time between sample collections. The 24-hour concentrations were then read from the graph.

Cows receiving a daily intake of about 15 γ of iodine also demonstrated the seasonal effect. A chronological compilation of the data from July, 1954, to June, 1955, is presented (Table 4). These data show that the seasonal effect does not merely cause more of the I^{131} to appear in the milk but, more important, it changes the mode of handling of blood iodine by the mammary gland. In order to put this effect into numerical terms, the concentration of I^{131} in the milk 24 hours after dosing was divided by the concentration of I^{131} in the blood at this time. It was evident that the mammary gland actively concentrated iodine in milk during late spring.

The milk and blood concentration curves for Cow A are presented (Figure 1) for the seven-day experimental periods during January and May. All values for milk have been corrected for time of secretion, to prevent depicting a time-lag phenomenon. During January the milk and blood curves were nearly identical and diverged only during the last few days of the trial. Analysis at Day 1 showed that less than two per cent of the blood I^{131} was protein-bound. This fraction increased gradually during the trial until at Day 7, 48% of the activity was associated with the protein. If it is assumed that this I^{131} was unavailable for milk production, then this would explain the divergence of the milk and blood disappearance curves.

During May the milk concentration of I^{131} greatly exceeded that of the blood, but these amounts tended to converge somewhat toward the end of the experiment. In this trial the P.B. I^{131} rose from four to 38%.

Figure 2 is a similar plot of the data collected on Cow C. The low-iodine ration was probably responsible for the rapid appearance and large proportion

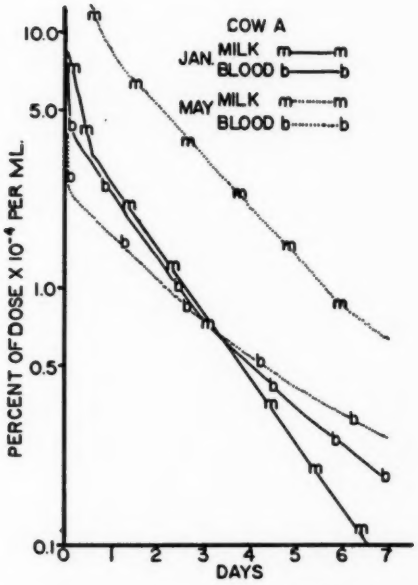

FIG. 1. Comparative concentrations of radioiodine in the blood and milk of Cow A at two different seasons.

(about 60%) of bound iodine in the blood, and resulted in a slow rate of disappearance of I^{131}. During May the concentration of radioiodine in milk exceeded that in the blood, as with Cow A. After the second day the protein-bound iodine accounted for nearly all of the blood I^{131}.

The feasibility of using daily doses of I^{131} to label all of the body iodine uniformly has been reported *(5)*. In those experiments it was found that milk and blood concentrations of I^{131} became nearly constant after seven days of oral doses in February, but 11 daily administrations were required to achieve this result in April. At the latter period, increased concentrations of I^{131} in the blood, milk, and thyroid were noted.

DISCUSSION

These experiments have indicated that as the season advanced from late winter to early summer a change occurred in the metabolism of iodine by the dairy cow. This influence was so pronounced that it obscured the possible effects of stage of lactation or level of production on the amount of iodine appearing in the milk. Iodine content of the thyroid is low *(9)* during late winter, possibly due to an increased rate of secretion of thyroxine at this time *(3)*. The thyroid is very active, as evidenced by a high uptake of I^{131} (Table 3). The metabolic need for iodine, presumably for thyroid hormone production, decreases the amount of inorganic iodine in the blood, which accounts, in part,

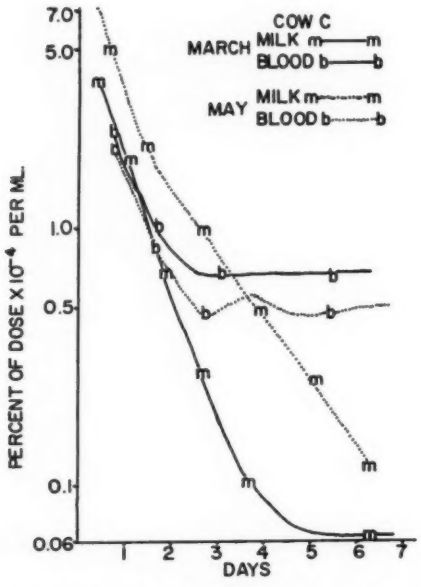

FIG. 2. Comparative concentrations of radioiodine in the blood and milk of Cow C at two different seasons.

for the low milk I^{131} values seen at this time. As shown by these data (Tables 2, 3, 4), with the onset of spring the concentration of I^{131} increased in the blood and milk, and the thyroid tended to accumulate more radioiodine than in winter. By May, or late spring, the rate of accumulation of new iodine (Table 3) by the thyroid had decreased, possibly because of near saturation, while the I^{131} concentration in the milk increased. The same effect was observed in the July but not in the October experiment.

These results are supported by the findings of Matthews *et al.* (*6*), in Ohio, that cow's milk is lowest in iodine in late spring and highest in August. The thyroid data agree well with the findings of Seiden and Fenger *(9)*, that cattle thyroids contain three times as much iodine from June to November as from December to May.

The rise in milk I^{131} with the advance of season was not due solely to the lowered metabolic need for thyroid hormone production. The data (Table 4) clearly indicate that the mammary gland actively concentrated iodine to put into the milk during the spring-summer season; whereas, it had little tendency to do this during the winter. The explanation for this activity of the mammary gland is not apparent from these experiments. This concentrating ability is not so marked as that observed in the human and in the dog. Both of these species show an ability to concentrate iodine in milk by a factor of 30 over the blood iodine *(1, 10)*, seemingly independent of season. Cow A showed a milk

level of I^{131} in May that was only five times that of the blood, whereas in January milk and blood levels of I^{131} were equal.

SUMMARY

A significant effect of season on the metabolism of iodine by the dairy cow was demonstrated, using I^{131} in single doses and in a continuous daily dosing study. With these techniques it was determined that there is a stimulus that coincides with the onset of spring which causes the blood and milk levels of iodine^{131} to increase. The mammary gland also exhibited a marked tendency to concentrate iodine in the milk with the onset of the seasonal effect.

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DETERMINATION OF THE SOLIDS IN MILK BY A LACTOMETRIC METHOD AT 102° F.[1]

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A formula is presented for calculating the solids in milk from the fat percentage and the lactometer reading at 102° F. The average deviation from the gravimetric method was + 0.05% total solids and the standard error for 99 samples of individual cow's milk was ± 0.13%. For 101 samples of herd milk, the average deviation was − 0.006% and the standard error ± 0.09% total solids. *Editor.*

Formulas for calculating the percentage of total solids and solids-not-fat in milk have been studied in the United States and abroad for many years. More than 60 different equations for computing the relation between the fat content and specific gravity and the solids of milk have been submitted by investigators. These equations appear to give reliable estimates of milk solids only when applied to data from which they were derived. Many researchers have substituted constants and corrections in the basic equations, in order to make the computed values agree with the experimental ones. The different constants, corrections, and divergencies in the calculated values have caused confusion and doubt concerning the fundamental reliability of the method. Comprehensive reviews and studies of the various formulas and techniques are available *(7, 8, 9, 10, 20)*.

The lag in the development of a more satisfactory method may be due to the emphasis that has been placed for many years on the fat content of milk as the basis for paying producers. The growing importance of the nonfat milk solids in the economy of the dairy industry has made it urgent to have a quick and economical method for determining milk solids, which would be comparable in speed and accuracy to the Babcock test for fat. Hence, an intensive study was made to develop a practical method for determining milk solids, which would avoid the difficulties previously encountered.

This method has been reported *(18)*. In the present paper, the sources of error, experimental results, and lactometer design are considered in more detail.

FORMULA FOR CALCULATION OF MILK SOLIDS

An algebraic equation was derived from the theoretical equation *(19)* for the calculation of the solids from the lactometer and the Babcock fat determinations. The constant (0.31) of the original formula was increased to 0.4 to compensate

Received for publication April 2, 1956.

[1] This work was done with funds delegated by the Agricultural Marketing Service.

for a change in the calibration of the lactometers, which was made later by the National Bureau of Standards:

$$\% \text{ T.S.} = 1.33\ F + \frac{273L}{L + 1000} - 0.40$$

F = Babcock fat % L = Lactometer reading in degrees

The nonfat solids may be found by using 0.33 F instead of 1.33 F, or by subtraction of the fat percentage from the total solids percentage. The total solids of skimmilk samples can be calculated by dropping the constant (0.40) from the formula.

SOURCES OF ERROR

A review of previous methods has indicated that unsatisfactory results are owing largely to two causes. One error is the indefinite physical state of milk fat at 60° F., the temperature in general use for the lactometer readings. The uncertain degree of solidification of fat at this temperature makes it unlikely that consistent values can be obtained. That the fat should be in a uniform physical state for the test has been known for many years, but solution of the problem has been slow. Various workers have preheated the milk samples to 104° F., or even as high as 113° F., and then cooled them to 60° or 86° F. before reading the lactometer *(2, 11, 17)*.

A second error is the general use of unsuitably designed and inaccurately calibrated lactometers. Many in use have not been tested for accuracy and have not been satisfactorily checked; few have been certified for accuracy by the National Bureau of Standards.

The literature indicates that it is common practice to check the calibration of lactometers in solutions of acid, salts, or sugars, which have a surface tension considerably higher than that of milk. A survey of commercial lactometers indicated none was entirely suitable for accurate milk-solids determinations. The practical types were so coarsely graduated that readings of the required precision could not be made, and the finely graduated instruments were too fragile for general use. Almost all types examined were found to have inaccurate scales, compared with lactometers which had been calibrated by the National Bureau of Standards. Invariably, no mention is made of any correction for differences of surface tension and of the meniscus, which can cause appreciable errors in evaluating the various formulas for calculating milk solids. These difficulties can be avoided by checking the calibration of a lactometer with milk, by means of specific gravity bottles. The use of normal milk is impractical owing to fat rising, but skimmilk and homogenized milk are suitable.

Since the percentage of fat has a marked effect on the specific gravity of milk, it is used with the lactometer reading in formulas for the calculation of total solids. It is known that Babcock tests for fat may differ by 0.1% or more in comparative tests by different technicians *(4, 12)*. An error of 0.1% results in a difference of 0.13% in the calculated total solids, and it has been held that an excessive degree of precision in the lactometer reading is not justified. The necessity for close temperature regulation has been ignored for the same reason.

Consequently, some researchers have questioned the need for the sensitive type of lactometer with a large bulb and a scale reading closer than 0.5 of a lactometer degree. Herrington *(8)* has pointed out that an error of 0.08° Quevenne is as important as an error of 0.1% of fat in the calculation of the nonfat solids of milk.

Roeder *(16)* made a comprehensive study of the sources of error in the total solids method in which the lactometer and Gerber fat values were used with the Fleischmann formula. The largest single source of error was attributed to variations in the specific gravity of the milk constituents. He estimated that a total possible theoretical error of ± 0.318% exists, but that the limits of error must be assumed to be between ± 0.15% total solids to allow for the possibility of a compensation of errors.

DEVELOPMENT OF METHOD

Consideration of the temperature problem has led to the conclusion that the lactometer method can be made more practical by eliminating the cooling step, which has been recommended by others. The new procedure consists of heating the milk samples to a temperature at which the milk fat is in a uniform liquid state and then reading the lactometer at this temperature. The temperature must be high enough to insure fat liquidity, but not so high that other physical changes might occur to affect the specific gravity of the milk. Experiments showed that consistently reproducible results can be obtained if the temperature is 102° F. (39° C.).

No provision was made for temperature variations and consequent corrections in the experimental work, because 102° F. is a minimum temperature and corrections could be a source of unnecessary error. The temperature can be readily maintained by immersing the lactometer cylinder in a water bath equipped with thermostatic control. However, for workers in the field who might want to use this method where no regulated water bath is at hand, it is suggested that the temperature be allowed to vary not more than two degrees from 102° F. for the reading. For each degree above 102° F., the lactometer reading should be corrected by adding 0.2 lactometer degree, and for each degree below 102° F. it should be corrected by subtracting 0.2 lactometer degree from the reading.

LACTOMETER DESIGN

The preliminary experimental work was carried out with a fragile type of commercial lactometer which was graduated to 0.1 lactometer degree at 60° F., and which had been calibrated by the National Bureau of Standards. When it was used at 102° F., it was necessary to apply a large correction [+ 6.6] for the difference in expansion of glass and milk over the range of 60° to 102° F. Consequently, a new lactometer was designed which is calibrated to read the specific gravity of milk at 102° F., referred to water at 102° F. from 25.6 and 37.4 lactometer degrees.

It was necessary to provide a large bulb in order to obtain an instrument with a sturdy stem and sensitivity of 0.2 degree (two in the fourth decimal place,

expressed as specific gravity). The design was in accordance with the equation for the dimensions of hydrometers derived at the National Bureau of Standards *(15)*. It is possible to read this lactometer to 0.1 degree if desired. A milk sample of about 10 oz. is required. This lactometer is commercially available[2] and the specifications are given in Figure 1.

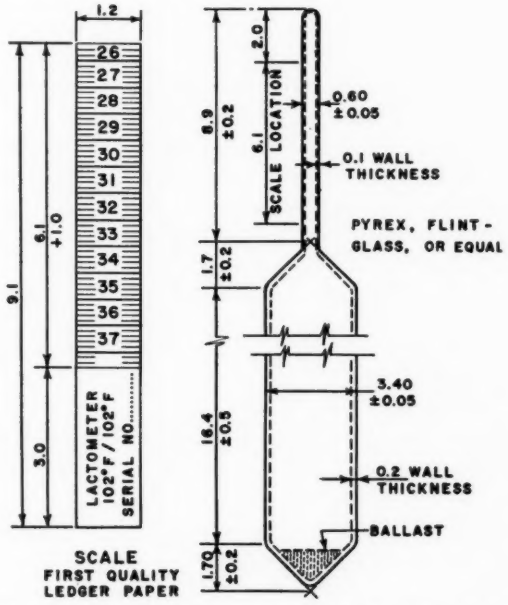

FIG. 1. Dimensions of 102° F. glass lactometer. All dimensions are in centimeters.

It is realized that use of this lactometer for testing the milks of a large number of individual cows in a herd would require much milk. Accordingly, the manufacturer cooperated in designing a smaller experimental model which appears to be suitable (it also has other uses, where the more sensitive instrument is not needed). This small lactometer requires only about 5 oz. of milk and is less susceptible to breakage.

The following dimensions in cm. are approximate, since certain tolerances are allowable for glas hydrometers: total length, 15; stem length, 6.5; stem diameter, 0.5; bulb diameter, 3.5, scale length, 0.23. The bulb displaces about 65 ml. of liquid.

The scale is designed for whole milk over the range of 25.5 to 33.5 degrees, the smallest division being 0.5 degree. Experiments have indicated that readings may be estimated to 0.25 degree.

[2] Catalog No. 22261, Taylor Instrument Companies, Rochester, N. Y. (Mention of this firm does not constitute endorsement of its products by the U. S. Department of Agriculture over similar items or products not mentioned.)

EXPERIMENTAL WORK

The experiments were conducted by rapidly heating the milk samples to about 102° F. in a few minutes in water at about 115° F., with occasional gentle shaking. The samples were then transferred to lactometer cylinders held in a water bath which was thermostatically set at 102° ± 0.3°F. Several readings were made, and in many experiments were checked against other lactometers. Duplicate readings checked to 0.1 lactometer degree. Experiments demonstrated that milk in good condition could be held in cylinders at 102° F. for over one-half hour with a change of only 0.1 degree in the lactometer reading, if gently stirred occasionally.

The Babcock fat tests were made according to the method in general use *(1, 13)*. Accurately calibrated bottles and pipettes were used. The 17.6-ml. pipettes were filled by adjusting the top of the milk surface to the graduation line, then allowed to drain for about 15 seconds before blowing out the last drop. A shaker was used to mix the milk and acid in the test bottles in many of the experiments, and during the summer the acid was cooled prior to use. Readings were made in duplicate and read to hundredths of a per cent fat by means of an illuminated reader with magnifier. Duplicate determinations agreed closely and were often checked by another observer.

Total solids determinations were made in duplicate, using the Mojonnier method and apparatus in which the samples were heated for 10 minutes in a vacuum oven regulated at 100° C. (212° F.) *(11)*. Generally, duplicate results agreed by 0.01 to 0.03% total solids, or closer.

Two hundred samples of milk, selected during all seasons of the year from about 25 herds in Maryland and Virginia, were used in plots of experimental results. The deviations of the percentage of calculated total solids from the percentage of gravimetric total solids were separately plotted for milk samples from two or more cows (see Figure 2) and for milk from individual cows (Figure 3).

The points compromise 101 mixed or herd milk samples and 99 samples from individual cows of six different breeds. The mixed milk experiments included 83 samples from about two to 14 cows and 18 samples from herds and dairy plants which represented from about 15 to 300 cows.

A cooperative research project on this F. lactometer method was conducted by the Dairy Department, College of Agriculture, University of Maryland, and through their courtesy it was possible to include in the calculations the results of 59 tests made on individual cows of the four major breeds *(3)*.[3]

It will be observed that the points indicating the deviation for milk from individual Holstein cows are mostly positive. Not as many cows are represented as would appear, however, since samples from two Holstein cows accounted for 12 of these positive deviations.

[3] This work was sponsored by the American Dairy Association.

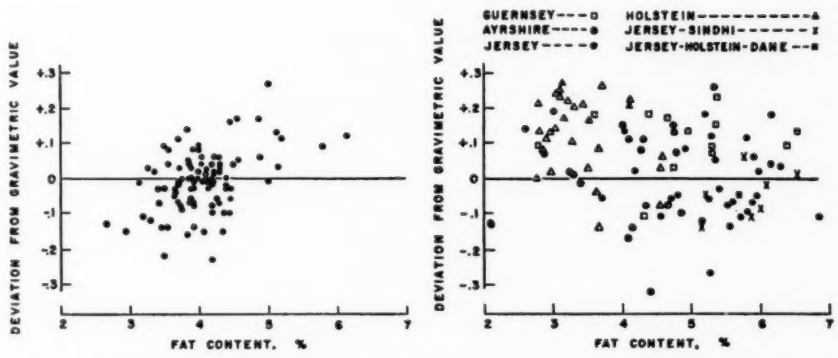

FIG. 2. Deviations of the calculated per cent of total solids from the gravimetric per cent of total solids for milk from two or more cows.

FIG. 3. Deviations of the calculated per cent of total solids from the gravimetric per cent of total solids for milk from individual cows.

Deviations of the calculated values from the gravimetric values for total solids in milk from individual cows were computed and the average deviation was + 0.05%. The standard error of estimate computed from these deviations (about the mean deviation) was ± 0.13. This measurement can be interpreted as meaning that about two-thirds of the calculated values could be expected to fall within + 0.05 ± 0.13% of the gravimetric total solids, or within from − 0.08 to + 0.18; about 95% of the calculated values could be expected to fall within + 0.05 ± 0.26% of the gravimetric values, or within from − 0.21 to + 0.31.

Similarly, for the mixed or herd milk samples, the average deviation was − 0.006% total solids, and the standard error of estimate was ± 0.091%. Consequently, about 95% of the calculated values on mixed milk could be expected to fall within − 0.006 ± 0.182% of the gravimetric values, or within from − 0.19 to + 0.18.

A linear least-squares equation was fitted to the gravimetric total solids value for samples from individual cows. This equation for calculating total solids from fat and lactometer values is: $T = 1.364\,F + 0.275\,L - 0.879$. This relationship, calculated from these particular data, agrees well with the theoretically derived equation.

The standard error of estimate of the percentage of total solids computed from this equation was ± 0.12.

The data for samples of herd milk were represented by the linear least-squares equation: $T = 1.254\,F + 0.271\,L - 0.261$.

This equation had a standard error of estimate of ± 0.08.

THE METHOD IN PRACTICE

Lactometer Test. Rapidly heat the milk sample (about one-third of a quart) to a temperature of approximately 102° F. by immersing the sample in warm

water (about 115° F.). Gently shake the sample at intervals to prevent overheating. The flask should be loosely stoppered, to reduce evaporation.

Transfer the sample with as little agitation as possible to a lactometer cylinder held in a water bath with the temperature thermostatically maintained at 102° F. The lactometer should be preheated for not less than three minutes before using. This can be done by immersing it in a cylinder of water held in in the same constant temperature bath as the milk sample. Remove the lactometer and wipe dry immediately prior to use.

Slowly immerse the lactometer in the milk sample. The reading is taken at the top of the meniscus after the lactometer comes to rest. It is important that the lactometer stem be clean and dry above the milk surface. Repeat readings can be made by withdrawing the lactometer just enough to enable the operator to wipe the stem clean with tissue or a soft cloth before slowly immersing it to the reading point.

Each lactometer degree on the scale is divided into 0.2 degree, and readings may be estimated readily to 0.1 degree. Successive readings should agree by 0.1 degree if the temperature remains constant. The temperature of the milk should be checked with an accurate thermometer at the time of reading.

Fat Test. The fat tests are conducted by the standard Babcock method or its equivalent *(1)*. The surface of the milk in the 17.6-ml. pipette should be adjusted to the graduation line. The amount of fat should be read to the nearest 0.05%. In applying the formula to skimmilk, the fat content was estimated by the American Association test *(5)*, a modification of the Babcock fat test.

General Suggestions. A large number of determinations can be carried out rapidly by using several lactometer cylinders in a constant temperature bath. The cylinders of milk can be held at this temperature for periods of about one hour with only negligible change in the specific gravity, provided that the milk is gently stirred before immersing the lactometer. This long holding method is not suitable for use with old milk, which tends to become rancid or to oil off.

When the milk samples are rather uniform in fat content, it is not necessary, for practical work, to wash and dry the lactometer between each measurement, because the error due to residual milk adhering to the lactometer from one sample to another is negligible *(16)*. However, the instrument should be rapidly transfered and then only the stem need be wiped dry.

Grant *(6)*, in using this lactometer method, found that composite samples could be preserved for eight days with only negligible changes in the specific gravity. The samples were refrigerated at about 40° F. after the addition of 0.5 ml. of formalin (40% solution) per pint of milk.

SUMMARY

A method is presented for the calculation by a formula of the per cent of solids in milk from the percentage of fat, and the lactometer reading made at the the temperature of 102° F.

A lactometer designed for use at 102° F. is described.

The results on 200 samples of milk from about 25 herds show the deviation of the calculated total solids from the gravimetric (Mojonnier) total solids, and are presented graphically. The average deviation was + 0.05% total solids and the standard error of estimate was ± 0.13 for 99 samples of milk from individual cows. The average deviation was − 0.006% total solids and the standard error of estimate was ± 0.09% for 101 samples of mixed or herd milk.

ACKNOWLEDGMENTS

The author wishes to acknowledge the cooperation and assistance of the following individuals: E. O. Whittier, who advised concerning the design of the lactometer and the preparation of the formula; the late G. E. Holm, who made many helpful suggestions; and F. P. Hanrahan, who aided in designing the instrument. L. F. Herrman and Elsie D. Anderson of the Marketing Research Division, Agricultural Marketing Service, cooperated in the statistical analysis of the experimental results. N. E. Yongue of the Chemical Laboratory of the District of Columbia Health Department supplied numerous samples of platform milk from this milk-shed. E. A. Capelle of the Taylor Instrument Companies, Rochester, N. Y., was very helpful in providing experimental models of the new lactometer. C. T. Collett of the Mechanics Division of the National Bureau of Standards gave full cooperation in the calibration of the lactometer.

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INSOLUBLE SCUM-LIKE MATERIALS ON RECONSTITUTED WHOLE MILK POWDERS[1]

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Solubility of whole milk powder is related to free fat which decreases during storage at 85° F., but is unaffected at 45° F. The relative insolubility of powder at the higher temperature is probably due to a complex of free fat and protein, with possible complications due to calcium. *Editor.*

If whole milk powder is reconstituted soon after manufacture, visibly the the product can hardly be distinguished from fresh homogenized milk. But if reconstitution is delayed, a deterioration in solubility may be observed within a few days, especially if the powder is held at about 85° F. Typical solubility defects described by Wilster *(6)* are, formation of surface scum, adherence to the container of undispersed particles, and appearance of distinct top and bottom layers after reconstituted milk has been undisturbed for several hours in the refrigerator.

Surface scum coats the sides of the container, giving reconstituted milk a displeasing appearance. This defect may be delayed several months if the powder is refrigerated. The defect is not found in skimmilk powders.

This investigation of scum development was undertaken to obtain information that might lead to increasing the storage life of whole milk powder. Attention was directed to the free-fat content as it might influence the formation of an insoluble fat-protein complex in milk powder.

EXPERIMENTAL PROCEDURE

Powder manufacture. Raw milk obtained from the College herd was preheated in a 30-gal. pasteurizer at various temperature-time relationships (170° F. for 1 to 30 minutes, to 190° F. for 1 to 5 minutes). The milk was then cooled to 100° F. and separated, and the nonfat milk was condensed in a Rogers 16-inch vacuum pan to approximately 36% total solids. Concentrate was brought to desired fat level by adding cream and then heating to 130° to 140° F. before homogenizing twice in a Creamery Package Multi-Flo Homogenizer at 2,000 p.s.i. The concentrate was cooled to 80° F. and spray-dried at a rate of about

Received for publication August 6, 1956.

[1] Scientific Paper No. 1521, Washington Agricultural Experiment Stations, Pullman. Project No. 783.

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80 ml. per minute. The custom-built experimental drier was a stainless steel vertical cone, with the drying air and atomized milk flowing concurrently downward. The concentrated milk entering the drier with a four-foot gravity head was atomized through a spray nozzle of 1-mm. diameter, centered within an air outlet 2 mm. in diameter, through which air at 60-lb. pressure was forced. The drying air entered at a temperature of from 250° to 270° F. The moist air was drawn from the drier at about 140° to 160° F. through six Rogers-type cloth bags. The powder was shaken from the bags at 15-minute intervals and collected from the cone at the end of each drying period (about one hour). The resulting powder was air-packed in No. 2 flat cans, hermetically sealed, divided between two storage treatments, 45° and 85° F. Occasionally a can was stored at 100° F.

The fat content of each batch of powder was determined by the Roese-Gottlieb ether-extraction method *(1)*. Free-fat content of the powder was determined by extraction in a Soxhlet continuous fat extractor. Petroleum ether was used and extraction continued for 16 hours at room temperature.

Isolation and chemical analyses of scum. Ninety-six grams of powder were added to 600 ml. distilled water (70° to 80° F.) in a 1-l. separatory funnel and reconstituted by shaking the funnel vertically for two minutes. The milk was then slowly drained out, leaving the scum on the wall of the funnel. The scum was redispersed in about 400 ml. distilled ice-water and allowed to rise to the surface, after which the water was slowly drained out. The washed scum was then removed from the funnel with about 50 ml. distilled water (120° to 140° F.), and collected. The procedure was repeated with a second batch of the same powder, and the combined scums were made up to volume in a 100-ml. volumetric flask. The flask was placed in a water bath (140° F.) until the scum was completely dispersed.

The following determinations were carried out in duplicate on the dispersed scum: Total solids, by using a 10-ml. aliquot and drying in an air oven at 208° F. for eight hours; protein, by using a 10-ml. aliquot by the standard Kjeldahl method *(1)*; fat, by using a 10-ml. aliquot by the Roese-Gottlieb ether-extraction method *(1)*; ash, by using the aqueous phase following fat extraction, according to the standard method *(1)*; calcium, by following dry-ashing by the standard ammonium oxalate method *(1)*; phosphorus, by following dry-ashing by the standard method *(1)*; carbohydrate, by difference; saponification number, by modifying the standard method *(1)* to accommodate small quantities of fat — refluxing the fat for 75 minutes with 0.35 *N* alcoholic KOH and titrating with 0.2 *N* HCl; iodine number, by modifying the standard Wijs method *(1)* — dissolving the fat in 5 ml. CCl_4, adding 15 ml. iodine solution, placing the flask in the dark for 30 minutes, adding 10 ml. 15% KI and 50 ml. distilled water, and titrating the solution with 0.1 *N* $Na_2S_2O_3$ with starch as an indicator; melting point of fat, determined by the capillary tube method *(1)*, nitrogen distribution, determined according to the method of Shahani and Sommer *(5)*.

Method for estimating degree of scum formation in reconstituted powder. The procedure for isolating scum described above was modified as follows: 16 gm.

milk powder were reconstituted with 100 ml. distilled water in a 125-ml. pear-shaped separatory funnel. The milk was slowly drained from the separatory funnel, which was then allowed to stand with open stopcock for an additional two minutes, for complete drainage. The scum was quantitatively transferred from the funnel to a test tube by rinsing the funnel with exactly 20 ml. 0.2 *N* NH_4OH. After the test tube was shaken briefly to dissolve the scum, total solids were determined in duplicate on five ml. of the solution by drying in an air oven.

RESULTS AND DISCUSSION

Free-fat content of milk powder. Free-fat content was determined in more than 200 samples of milk powder. With few exceptions, powder stored at 85° F. contained less free fat than did the same powder stored at 45° F. Figure 1 shows the effect of storage temperature on the free-fat content of three typical powders differing only in fat content. These powders were removed from storage and held at room temperature for 48 hours before extraction, to minimize any physical differences in the state of the fat. The rapid decrease in free fat when powders were held at 85° F., reaching a minimum as measured at 14 days, is not clearly understood. Previous workers have shown that free fat is attached to the surface of the powder granule *(1)*. It is possible that at 85° F. the free fat in the liquid state may be absorbed within the granule proper and thus removed from contact with solvent. It is difficult, however, to account for the decrease in free fat by absorption alone, since powder could also absorb the solvent. There is, therefore, the possibility that under these conditions, free fat enters into a fat-protein complex, which is stable to solvent extraction.

Also, it can be seen (Figure 1) that the initial amount of free fat, as well as the amount of decrease at 85° F. storage, is related to the total fat content of the powder. In Figure 2, A, the sharp increase in free fat that sets in at about 26% total fat is in excellent agreement with data reported by Holm *et al. (2)*. A highly significant linear relationship ($P < 0.01$) was found between total fat and free fat when powders that contained from 25.1 to 35.0% fat were held at 45° F. This relationship was not significant ($P < 0.05$) when powders contained from 5.0 to 15.0 or from 15.1 to 25.0% fat.

Relationship between free fat and scum. Effect of total fat content on the extent of scum development in powders reconstituted after storage at 45° and 85° F. is presented (Figure 2, B). Extensive scum development was found when the powders containing more than 26% fat were held at 85° F., the curve being similar to that for initial free-fat content of powder (compare A and B of Figure 2). Since this defect is concerned mainly with powders held at the elevated temperature, it is possible that the formation of scum involves the same mechanism by which the free-fat content of powder diminishes at that temperature (Figure 1). This mechanism by which fat combines with other milk constituents to form an insoluble material is not clearly understood. It may be similar to the absorption of fat during the precipitation of casein from milk by any of several methods: acid, rennet, alcohol, and salt. Free fat decreases most rapidly dur-

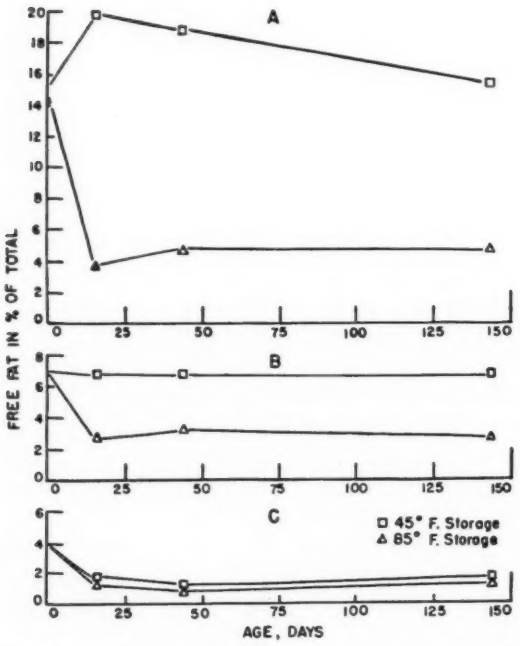

FIG. 1. Influence of total fat and storage temperature on the free-fat content of milk powder. A—34.4% fat, B—28.6% fat, C—13.0% fat.

ing the first few days of storage; whereas, scum development proceeds gradually and is not recognized until after several weeks of storage. This indicates that the reaction takes place in more than one step.

Chemical analysis of the scum. Results (Table 1) show that the scum always contained a larger percentage of fat and protein, together with a smaller per-

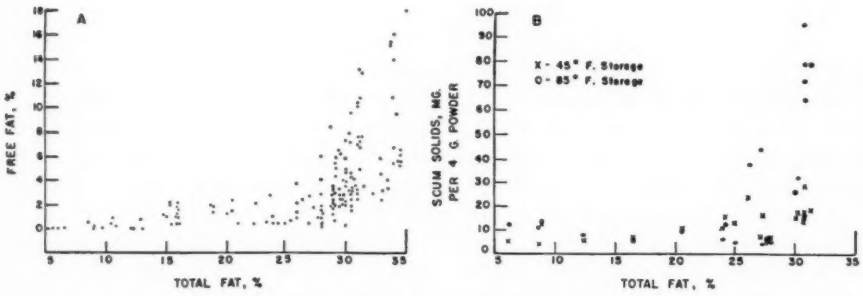

FIG. 2. A—Effect of total fat on free-fat content of milk powder held at 45° F.; B—and on quantity of scum solids isolated from milk reconstituted from powder held at 45° F. and at 85° F.

TABLE 1

Average chemical analyses of ten milk powders stored at room temperature

Determination	Scum fraction solids	Normal fraction solids
Total protein *(%)*	33.0	26.2
Fat *(%)*	48.2	27.4
Saponification No.	223	223
Iodine No.	30.4	32.6
Melting point *(° F.)*	95.0	85.1
Ash *(%)*	4.2	5.8
Calcium *(% of ash)*	42.9	19.0
Phosphorus *(% of ash)*	16.6	12.0
Carbohydrate *(% by diff.)*	14.6	40.6

centage of ash and carbohydrate, than did the soluble fraction. Saponification numbers of the fats from each fraction were identical, showing similar molecular weights. Fat isolated from the scum had a consistently lower iodine number and higher melting point. It is not known whether the more highly saturated fat found in the scum fraction might have been the result of fat oxidation.

Nitrogen distribution in the two fractions is summarized (Table 2), in which data are arranged in the order of increasing preheat treatment. Between 82 and 95% of the nitrogen present in the scum fraction consisted of casein plus denatured whey protein. These values increased as the preheat treatment of the original milk was increased. The nonscum fraction contained less casein plus denatured whey protein in each case than did the scum fraction from the same powder. There was no parallel increase with heat treatment. Alcohol stability of the two protein fractions was determined in the following manner: A dispersion of each fraction in water was prepared so that the protein content was 11 mg. per milliliter. Then alcohol was added to each until the final concentration was 43%. Upon centrifuging these mixtures, 85% of the scum protein was precipitated; whereas, only 8% of the nonscum protein was destabilized by the alcohol.

Manus and Ashworth *(3)* noted that powders made from milk receiving low preheat treatment did not decrease appreciably in solubility when stored at 100° F., but that a continuous decrease in solubility resulted in powders that had high preheat treatment. Wilster *(6)* reduced scum development by preventing excessive heating of powder in the drying chamber. It is possible that milk protein rendered unstable during drying is further denatured when the milk powder is stored at 85° F., this unstable protein being more likely to complex with the free fat present.

SUMMARY

A method is described by which it is possible to measure the extent of formation of an insoluble scum on the surface of reconstituted whole milk.

Extensive scum development was found on milk from powders stored at 85° F., but little scum was found when powders had been held at 45° F. The

TABLE 2

Nitrogen distribution in scum and nonscum fractions of milk powder reconstituted after storage at room temperature for seven years

Powder No.	Preheat treatment for 20 min.	Scum fraction				Nonscum fraction			
		Non-casein N	Proteose-peptone + NPN	Undenatured whey protein N	Casein + denatured whey protein N	Non-casein N	Proteose-peptone + NPN	Undenatured whey protein N	Casein + denatured whey protein N
	(° F.)	% [a]							
220	140	18.0	5.8	12.2	82.0	23.2	11.6	11.6	76.8
240	140	10.2	8.0	2.2	89.8	22.2	9.7	12.3	77.8
221	150	7.3	7.9	0.0	92.7	22.1	9.9	12.2	77.9
241	150	10.0	5.8	4.2	90.0	19.2	10.5	8.7	80.8
231	160	3.6	3.3	0.3	96.4	17.8	8.6	9.2	82.2
242	160	6.1	——	——	93.9	18.3	8.9	9.4	81.7
230	170	5.0	6.0	0.0	95.0	12.9	9.6	3.3	87.1

[a] Calculated as per cent of the total nitrogen.

free-fat content of powder rapidly decreased during storage at 85° F. There was no appreciable change in free-fat content when the powder was held at 45° F.

Scum development was related to the initial free-fat content of powder. It was postulated that development of this defect is the result of a fat-protein complex involving free fat and unstable protein, with possible involvement of calcium. Fat isolated from scum had a higher melting point and was more saturated than the remaining fat.

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SOME FACTORS AFFECTING THE FREEZING POINT OF MILK[1]

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Careless flushing of water through HTST pasteurizers, storage tanks, and other equipment was responsible for high freezing points in processed milk. Processing procedures did not affect the freezing point of milk. A maximum standard of –0.530° C. was satisfactory for milk. *Editor.*

The freezing point of milk has served for over 30 years as an official method *(2)* for detecting adulteration of milk with added water. Current investigations *(4, 7, 10)* have indicated that the present standard of − 0.550° C. is too low for the average freezing point of milk, and that average values frequently fall between − 0.535° C. and − 0.545° C. for milk of authentic origin, with still higher values for commercial market milk.

Observations that commercial milk had unexpectedly high freezing points, and attention toward determination of causes, formed the basis for this study. The major purposes of this study were to determine the mean freezing points of *(a)* milk from individual cows and herds, *(b)* commercial market milk, and *(c)* to study the factors affecting the freezing point of milk during processing operations at dairy plants.

METHODS AND PROCEDURE

A modified Hortvet cryoscope *(6)*, with a mechanical agitator to stir the samples and a mechanical tapping device for the thermometer, was used to determine the freezing point of the milks. Except for this improved apparatus, the official method *(2)* was followed in all other details. Duplicate or more tests were made on each sample until agreement within 0.002° C. was obtained. Calculation of per cent added water was made by the Association of Official Agricultural Chemists (A.O.A.C.) equation:

$$\% \text{ added water} = \frac{\text{F. P. of authentic milk} - \text{observed F. P.}}{\text{F. P. of authentic milk}} \times 100$$

(F. P. = Freezing point)

Received for publication October 9, 1956.

[1] Article No. 7-56 of the Department of Dairy Technology. Supported in part by the Ohio Dairy Products Research Fund. Journal Article No. 70-56 of the Ohio Agricultural Experiment Station.

Calculations were made also by the Elsdon and Stubbs *(5)* equation, which differs from that of the A.O.A.C. only by substituting (100 − % Total Solids) for the 100.

Fat content of the milk was determined in duplicate by the standard Babcock method *(2)* for regular milk and the modified Babcock test *(8)* for homogenized milk.

Total solids determinations were made in duplicate by the Mojonnier method *(8)* and/or estimated by the following formula from duplicate lactometer readings, employing the Taylor-Watson Pattern Lactometer *(11)*:

$$\text{T. S.} = 1.33\ \text{F} + 273\ \text{L}/(\text{L} + 100) - 0.40$$

where *F* is fat per cent and *L* is lactometer degrees at 102° F.

Milk samples were collected as indicated in the various experiments at the dairy barn from individual cows and herds, from the regular retail market, and from commercial dairy plants at various points during processing. All samples were cooled rapidly in ice water and taken immediately to the laboratory for analysis within 24 hours.

EXPERIMENTAL RESULTS

Relationship of freezing point to added water: Preliminary trials on 20 milk samples established the reliability of the freezing point depression method to detect as little as 1% added water when various amounts were added, unknown to the analyst.

Freezing point of raw milk, from individual cows and herds, producers' supply and storage tanks at plants: Both individual and composite samples were taken from cows of five different breeds. Colostrum and mastitis milk were excluded from the experiment as being abnormal milk. All samples from individual cows represent those yielding more than 15 lb. of milk per milking.

To determine the freezing point of milk as received at the dairy plants from individual can-shipping producers, 108 samples were collected at one large and two small dairies. At least three of each ten producers selected at the large dairy delivered more than 300 pounds daily. Samples were obtained from practically all shippers' milk at the two small dairies. The freezing points of milk in the raw milk storage tanks were compared with those of the milk from the producers supplying each tank. Freezing point results are shown (Table 1).

The average freezing point for 44 samples from individual cows was − 0.546° C. The range was from − 0.533° C. to − 0.560° C. Milk from Ayrshire herds showed the highest average freezing point in these trials (−0.541° C.). Milks from herds of the other four breeds had a fairly close range for average freezing point at − 0.546° C. to − 0.550° C. The average freezing point of 15 composite samples of all herds was − 0.546° C., and the extreme range for individual analyses was from − 0.540° C. to − 0.555° C.

The average freezing point of the milks from herds and individual cows was − 0.546° C. This compares quite closely with the standard of − 0.550° C. of the A.O.A.C. official method *(2)*.

TABLE 1

Freezing point of raw milk, from individual cows and herds, producers' supply, and storage tanks at plants

Source of sample	Trials	Freezing point	
		Av.	Range
	No.	*−0° C.*	*−0° C.*
Individual cows by breed			
Holstein	8	.548	.539 - .557
Jersey	9	.550	.543 - .557
Ayrshire	9	.541	.533 - .547
Guernsey	11	.546	.538 - .553
Brown Swiss	7	.550	.546 - .560
Average, all cows	44	.546	.533 - .560
Herds by breed			
Holstein	3	.546	.542 - .549
Jersey	3	.548	.547 - .548
Ayrshire	3	.542	.540 - .543
Guernsey	3	.546	.543 - .549
Brown Swiss	3	.550	.546 - .555
Average, all breeds	15	.546	.540 - .555
Raw Milk Receiving			
Weigh tank	108	.538	.469 - .552
Storage tank	10	.533	.523 - .541

The average freezing point of milk from 108 producers was −0.538° C. (2.1% added water); whereas, that from the corresponding milk in ten storage tanks was −0.533° C. This increase of 0.005° C. indicates 1.0% additional added water at this particular step. There were only seven of 108 producer samples (6.5%) with a freezing point above −0.531° C. (one was −0.469° C.). In comparison, samples from three of the ten storage tanks which contained the same producer milk showed freezing points above −0.531° C.

Commercial fluid milk survey. Fifty-three samples of regular and homogenized milk from 13 dairies were collected at random in both paper and glass from the retail outlets in a large Ohio city during May and November, 1955. Combined results are presented (Table 2). No essential difference was found in the freezing points of milks collected during these two periods.

TABLE 2

Freezing points of commercial fluid milk obtained from retail outlets

Type of sample	Trials	Freezing point	
		Av.	Range
	No.	*−0° C.*	*−0° C.*
All samples	53	.530	.437 - .549
Regular and homogenized (paired from same dairy)			
Regular	24	.534	.495 - .549
Homogenized	24	.531	.489 - .542
Paper and glass			
Paper	29	.533	.437 - .549
Glass	24	.526	.489 - .544

The average freezing point for all 53 samples was − 0.530° C. The range of freezing point was from − 0.437° C. to − 0.549° C. Freezing points of 38 (72%) samples fell within the range of − 0.531° C. to − 0.550° C., which represents the 3% tolerance allowed for added water. One sample exhibited an extremely high freezing point of − 0.437° C. (indicating the presence of 20.5% of added water) and the other 14 samples had freezing points over a wide range between − 0.489° C. to − 0.530° C. (11.1% to 3.6% added water).

The average freezing point of the regular milk was lower than that of homogenized milk from the same dairy by 0.030° C., which may be considered an insignificant difference.

Milk in paper containers (29 samples) averaged 0.007° C. lower in freezing point than milk in glass bottles (24 samples), showing − 0.533° C. and − 0.526° C., respectively. Only four of 29 milks in paper containers exhibited freezing points higher than − 0.530° C., whereas ten of 24 milks in glass containers had freezing points above − 0.530° C.

The freezing point of milk as affected by dairy plant processing factors: Additional experiments were designed to determine the effect of the various processes and practices on the freezing point of the milk supply in five dairy plants under commercial conditions of operation. In these, the same milk supply was followed through various operations, with samples being taken at selected points to determine the effect of the operations per se on the freezing point.

The results (Series 1, Table 3) indicate that standardization and clarification had no significant effect on the freezing point of milk, and (Series 2) that pasteurization and homogenization had no more than a slight effect, if any, on increasing the freezing point of milk.

In Series 3 of the trials, by the time the milk had reached the surge tank, its freezing point had increased on the average from − 0.525° C. to − 0.514° C. No further change in freezing point was observed for milk after leaving the surge tank, and filler, and after being bottled.

In certain operations, the sequence of bottling had a marked relationship with the freezing points of the milk. In this connection, the results (Series 4, Table 3) indicate a significant change in average freezing points, from − 0.527° C. for the raw milk to − 0.514° C., − 0.519° C., and − 0.512° C. for the milk bottled at the beginning, middle, and last stages of the process.

Results (Series 5, Table 3) show that the freezing points of bottled milk in both paper and glass averaged higher than those from the raw milk storage tank by 0.005° C. and 0.010° C., respectively, which correspond approximately to 1.0% and 2.0% added water in these operations. The freezing points of the bottled pasteurized milk were higher than those of the original supply in ten of the 11 trials. The increase was marked in six of them.

Observations at one dairy revealed a marked increase in the freezing point (0.010° C.) of the milk being processed by the HTST system, owing to water (approximately 2.0%) being added to the balance tank to flush out milk near the end of the operation. In another case, water was used to rinse the milk from

TABLE 3

Freezing point of milk as affected by dairy plant processing factors

Source of sample	Plants	Samples	Freezing point	
			Average	Range
	(No.)	*(No.)*	*(−0° C.)*	*(−0° C.)*
Series 1				
Standardization and clarification	1			
Storage tank (control)		2	.536	.533 - .539
Standardized		2	.534	.530 - .538
Clarified		2	.535	.531 - .538
Series 2				
Pasteurization and homogenization	5			
Storage tank (control)		7	.532	.521 - .540
Pasteurized		3	.528	.522 - .534
Homogenized		6	.530	.522 - .534
Series 3				
Filling (ten minutes after bottling began)	2			
Homogenized (control)		3	.525	.522 - .530
Surge tank		2	.514	.507 - .520
Filler		3	.515	.508 - .520
Glass bottle		3	.513	.504 - .517
Series 4				
Variations during the bottling process	4			
Raw milk (control)		6	.527	.510 - .540
Beginning of bottling (immediate)		6	.514	.487 - .534
Middle of bottling		5	.519	.494 - .534
Last of bottling (last bottle)		3	.512	.485 - .534
Series 5				
Processing operations (over-all)	5			
Storage tank (control)		11	.527	.510 - .540
Packaged milk — paper		9	.522	.499 - .534
Packaged milk — glass		7	.517	.494 - .534

the storage tank and the rinsings flowed through the processing system and into the bottles. The freezing point of the bottled milk increased immediately by 0.022° C., indicating 4.0% added water.

Relationship of freezing point to solids-not-fat and total solids content of milk: Although detailed analytical data are not given, Table 4 shows a general relationship between the solids-not-fat and total solids content, and the freezing point of commercial milk and of milk to which various amounts of water were

TABLE 4

Relationship of freezing point to calculated added water, solids-not-fat, and total solids content of milk

Samples	Freezing point		Calculated added water by A. O. A. C. formula	Solids-not-fat	Total solids
	Range	Average			
(No.)	*(−0° C.)*		*(Av. %)*	*(Av. %)*	*(Av. %)*
20	.549 - .540	.542	1.5	8.90	13.08
35	.539 - .530	.535	2.7	8.87	12.77
23	.529 - .520	.525	4.5	8.67	12.60
15	.519 - .500	.510	7.3	8.61	12.33
6	.499 - .485	.492	10.5	8.09	11.65
5	.441 - .428	.436	20.7	7.51	11.25

added. This was true when the solids-not-fat content was determined by the Mojonnier and the lactometric method. The correlation, however, was not high enough and the solids-not-fat determination served only as a guide to indicate the possibility of adulteration with water. Individual deviations from the mean were so great that they resulted in rather broad-scatter diagrams.

DISCUSSION

In proving the reliability of the freezing point method, when employing mechanical devices and every precaution to insure accuracy, it was possible to differentiate between samples which varied by 1.0% added water, which was equivalent to a difference in freezing point of 0.0055° C., as calculated by the A.O.A.C. equation. However, the average freezing point differences for groups of samples probably are significant in an even lower order of magnitude.

In this study, the average freezing point of − 0.546° C. was obtained for authentic milk from herds and individual cows, as compared to − 0.550° C., the A.O.A.C. average. Allowing 3% added water by the A.O.A.C. formula, the highest freezing point permitted would be − 0.530° C. from the − 0.546° C. base; whereas, − 0.534° C. would be the limit, using − 0.550° C. as the standard. The range of freezing points from individual cows was − 0.533° C. to − 0.560° C. There was one sample (0.533° C.) which could be then classified as adulterated or border-line on the − 0.534° C. limit; yet it was authentic milk. This would indicate that the level of − 0.530° C. should serve as a more satisfactory maximum freezing point of unadulterated milk as recommended by Doan *(1)*. When this standard was used in this work, 94% of the producers' samples were classified as unadulterated, in contrast to only 87% by the A.O.A.C. standard; retail samples showed 72% and 57%, respectively, by the two standards.

If − 0.540° C. is used as a standard, as has been suggested by Shipe, Dahlberg, and Herrington *(10)* and Lampert *(7)*, and 3% tolerance is allowed, the milk which has a freezing point lower than − 0.524° C. is considered as unadulterated. No milk from herds exhibited freezing points higher than − 0.536° C. in this study.

A small percentage of samples (2%) of both producers' and processors' milk was noted to have between 10 to 20% added water. Aschaffenburg and Temple *(1)* in England found 93.7% of all producers' samples unadulterated (lower than − 0.530° C.) and 1% with more than 10% added water.

The Elsdon and Stubbs *(5)* formula, which applies a correction factor for total solids to the regular A.O.A.C. equation for freezing point, was found by Ystgaard *et al. (13)* to be superior. When the two formulas are compared in this study, the Elsdon and Stubbs equation tended to classify milk with a freezing point of − 0.531°C. or lower as unadulterated. This is in very close agreement with the − 0.530° C. recommended by Doan *(4)*.

Results of a survey of pasteurized market milk in eight cities in the United States made by Dahlberg *et al. (3)* showed that the freezing points of milk of all the plants were within the range of − 0.525° C. to − 0.555° C., with an average

of $-0.540°$ C. Seventy-three per cent of 135 samples showed freezing points below $-0.534°$ C., as compared to 57% in this study. A survey by Paley and Tzall *(9)* showed only 40% of the samples of bottled fluid milk to have freezing points within the tolerance of 3% added water.

Results of this study, made from samples taken from pipelines and equipment in dairy plants, revealed that properly executed processing operations such as standardization, clarification, homogenization, and pasteurization had no significant effect on freezing point, although some trials showed slight changes of 0.002° C. and 0.004° C. This is in agreement with Yongue *(12)*, who reported that good processing practices did not cause a serious change in freezing point of milk.

Careless practices which varied between dairies accounted for many of the high freezing-point findings. Among these were: *(a)* allowing an unknown amount of circulation water to mix with the milk at the start of an HTST operation, because a stop-watch was not employed; *(b)* similar watering at the end of the HTST operation, to force the last milk from the system; *(c)* rinsing down the foam in milk storage tanks with water prior to completion of bottling, and *(d)* leaving water in glass bottles and filling equipment prior to bottling.

SUMMARY AND CONCLUSIONS

The freezing points of 59 authentic herd and individual cows' milk averaged $-0.546°$ C., with a range of from $-0.533°$ C. to $-0.560°$ C. This difference from the standard $-0.550°$ C., established by A.O.A.C. as the normal freezing point, amounts to 0.7% equivalent added water.

The freezing points of 108 milks received at dairy plants from producers averaged $-0.538°$ C., and ranged from $-0.450°$ C. to $-0.550°$ C. This average indicates 2.1% added water when calculated by the A.O.A.C. equation, using $-0.550°$ C. as the standard. Ninety-three per cent of these samples were lower than $-0.530°$ C. The freezing points of these same milks after transfer into ten storage tanks averaged $-0.533°$ C., and ranged from $-0.523°$ C. to $-0.541°$ C. An additional 1% added water had occurred, making a total of 3.1%.

The average freezing point of 53 samples collected from retail outlets was $-0.530°$ C., and range was $-0.437°$ C. to $-0.549°$ C. The average indicates 3.6% added water, and only 57% of these samples were within the tolerance of 3% added water permitted by the official method, with $-0.550°$ C. as the standard.

Processing operations such as standardization, clarification, pasteurization, and homogenization when properly executed did not elevate the freezing point.

It was found that water was getting into the milk at the farm, in the receiving operation, and during the handling and processing of the milk within the plant.

A general relationship was shown between the freezing point and the solids-not-fat and total solids content of milk with varying amounts of water added.

The validity of the existing standards, equations for calculation of per cent added water, and desirability of proposed changes of standards are all discussed. One standard, a maximum freezing point of $-0.530°$ C., was substantiated as being satisfactory.

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THE SPECIFICITY OF MILK LIPASE. IV. PARTITION OF THE LIPASE SYSTEM IN MILK[1]

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Cow's milk contains at least two lipases that are present in the plasma of warm, freshly drawn milk. One, the membrane lipase, is irreversibly adsorbed on the fat globules as milk is cooled. It is abundant and active in milk from cows late in their lactation. The other one, plasma lipase, is associated with the caseinate system and it can be activated to produce lipolysis in milk. *Editor.*

In the previous papers of this series *(6, 7)* evidence was presented that indicates the presence in milk of multiple lipolytic enzymes that include both lipase and simple esterases. Since milk contains no simple esters to serve as substrate, and the use of esters other than milk fat for lipase determination in milk can not be justified *(6, 7)*, the presence of esterases is of only theoretical interest. Hydrolytic rancidity in milk results from hydrolysis of milk fat. A study of the lipase system itself in milk is, therefore, of fundamental and practical importance. A knowledge of the distribution of milk lipase or lipases among various fractions of milk is essential both for the isolation of the enzymes and an understanding of the mechanism of action of this enzyme system. The present paper deals with the identifying of different lipases on the basis of their partition among some milk components.

At present, the development of hydrolytic rancidity in milk is generally associated with certain "activation" treatments of raw milk: homogenization *(2)*, temperature changes *(13)*, shaking *(11)*, or agitation by air *(22)*, as in pipeline milking systems. However, in the early literature *(11, 12, 16, 17, 20)* and during recent years *(28)*, lipase activity has been reported in the milk of individual cows—especially those in late lactation and on dry feed—without the milk's being subjected to any apparent activation treatment other than aging. Milk susceptible to this lipolysis has been designated naturally rancid *(2)*, bitter milk of late lactation *(16)*, naturally active or naturally lipolytically active *(11, 20, 21, 23)*, and normally active *(28)*. Tarassuk *et al. (21, 23)* have referred to this lipolysis as "spontaneous," to distinguish it from "induced" lipolysis which requires activation. In this paper, milk that exhibits spontaneous lipolysis is referred to as "naturally active" or "spontaneous" milk.

Received for publication August 19, 1956.

[1] This study was supported in part by funds from The California Dairy Industry Advisory Board.

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Krukovsky and Sharp (*14*) observed that activation by shaking of naturally active milk did not result in greater lipolysis. They concluded that all milk contains sufficient lipase to produce rancidity upon activation, but that the lipase in milk from certain cows is in a "naturally activated state." Tarassuk and Richardson (*25*) reported evidence that the cooling of naturally active milk is necessary to initiate spontaneous lipolysis and, that after cooling, the lipolysis proceeds whether the milk is kept warm or cold. Cooling normal milk, however, is not essential for activation of induced lipolysis by homogenization or by shaking. Furthermore, the lipolysis in spontaneous milk is inhibited by mixing spontaneous milk with normal milk within an hour after milking, a phenomenon which is not due to dilution of the lipase concentration of spontaneous milk (*23*).

To explain the differences in the mode of occurrence of lipolysis, Tarassuk *et al. (20, 21, 23, 25)* have distinguished between the "naturally active lipase," involved in spontaneous lipolysis, and the "lipase of normal milk," involved in induced lipolysis. The necessity of cooling to initiate lipolysis in spontaneous milk was explained by Tarassuk and Jack *(24)* on the basis of irreversible adsorption of naturally active lipase on the fat-globule membrane, upon the cooling and aging of milk at 5° C. for half an hour or longer. Since little or no adsorption of lipase on the membrane material occurred upon cooling of normal milk under the same conditions, the lipase of normal milk was considered to remain in the plasma.

Only limited information is available on the distribution of lipase in normal milk. Dorner and Widmer (*2*), the first to demonstrate the activation of lipolysis by homogenization, also indicated that the lipase is associated with the casein fraction of milk. Further evidence that lipase is in the plasma of milk was provided by Pfeffer *et al.* (*18*), who also reported that separator slime has about three times as much activity as the original skimmilk. The high tributyrinase activity of separator slime has been shown recently by Nelson and Jezeski (*15*). Gould (*8*) used rennet whey as the source of lipase to demonstrate the influence of pH upon the lipolysis of a rennet whey-butterfat mixture.

The first part of this paper presents data that heretofore have not been published on the adsorption of naturally active lipase on the fat-globule membrane material when spontaneous milk is cooled. In the second part, the relative lipolytic activity of the membrane material and casein obtained from normal and weakly spontaneous milk toward different esters was compared with the relative lipolytic activity of the original skimmilks. Evidence for different lipolytic enzymes in milk was based on variations in relative activity with the fractionation of milk.

EXPERIMENTAL PROCEDURE

Freshly drawn, naturally active milk from selected individual cows and mixed normal milk were used as enzyme sources. The lipolytic activity of the naturally active milk was evaluated by determining the extent of lipolysis after immediate cooling and aging at from 0 to 5° C. for 24 hours or longer.

In the first part of this paper, lipolysis was followed by determining surface tension at 20° C. with the duNoüy Tensiometer *(23)* and the acid degree on churned fat [3] *(10)*; in the second part, the free fat acidity was determined by an extraction-titration method *(1)*.

The relative lipolytic activity of milk toward Tween-20, methyl butyrate, tributyrin, and milk fat was determined by the methods described previously *(5)*. Activity was expressed as units per ml. of milk, or per gm. of solids-not-fat (S. N. F.). To follow the purification of lipase obtained, the "specific activity" of milk fractions was determined. This is the concentration of enzyme expressed as units per gm. protein nitrogen. The purification or enrichment achieved was calculated by dividing the specific activity of the fractions by that of the original milk. The total Kjeldahl nitrogen, the protein nitrogen, and the casein nitrogen were determined by the procedure described by Rowland *(19)*. Total solids were determined by the Mojonnier method; and the moisture in the powder was determined by the A.O.A.C. method.

EXPERIMENTAL RESULTS

Adsorption of naturally active lipase on fat globule membrane by cooling of milk. Milk containing naturally active lipase in appreciable amounts exhibits a spontaneous lipolysis, with no involved treatment necessary for the development of rancidity. The only condition necessary to initiate the hydrolysis of fat is the cooling of the milk as initially reported in 1941 *(25)*. Additional data on spontaneous lipolysis since that time have fully substantiated the necessity of cooling for the initiation of lipolysis. Once the milk has been cooled the lipolysis proceeds, whether the milk is aged in cold or is rewarmed to 20, 30, or 37° C. for aging. Activation of lipolysis in naturally active milk by cooling is a distinctly different phenomenon from the lipolysis activation in normal milk by temperature activation treatment. In this case the milk must be cooled, warmed, and recooled *(13)*.

The effect of cooling naturally highly active milk within 10 min. after milking on the development of lipolysis is illustrated (Figure 1). A cooled sample (Curve II) tested organoleptically two hours after cooling was found rancid, and its surface tension had dropped from 50.2 dynes/cm. (before cooling) to 42.5 dynes/cm.[4] An uncooled sample did not go rancid on aging, as determined by organoleptic tests and surface tension data (Curve I). The acid values of fat were as follows: before cooling, 0.32; after aging for 36 hours (uncooled—I), 0.57; and (cooled—II), 9.65. It may be argued that growth of bacteria *(27)* in uncooled milk aged at 37° C. may have prevented lipolysis. However, the addition of formaldehyde as a preservative did not materially change the pattern of lipolysis of cooled and uncooled milks. Further, a sample of the same milk

[3] Milk or cream was heated at from 70° to 75° C. for 15 minutes prior to churning, to inactivate the lipase.

[4] Once milk has been cooled, there is an initial decrease in surface tension of about 3 dynes/cm. as measured at 20° C. Any further decrease would be due to lipolysis.

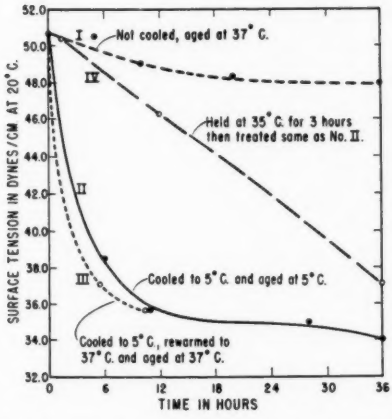
SURFACE TENSION IN DYNES/CM AT 20° C.
TIME IN HOURS
I Not cooled, aged at 37° C.
IV
Held at 35° C. for 3 hours then treated same as No. II.
II
III
Cooled to 5° C. and aged at 5° C.
Cooled to 5° C., rewarmed to 37° C. and aged at 37° C.

FIG. 1. Lipolysis in naturally lipolytically active milk.

cooled to 5° C., then rewarmed and aged at 37° C. (III), showed even greater rate of development of lipolysis than the sample aged in cold (II). Working with naturally active milk of various degrees of lipolytic activity and bacteria content, it was found that incubation of once-cooled milk at 37° C. does not always give a greater velocity of lipolysis. In milk of slight or moderate lipolytic activity, growth of organisms will inhibit or limit the extent of lipolysis. In milk of high lipolytic activity, the lipolysis will proceed, actually inhibiting the growth of organisms *(27)*.

Holding naturally active milk at or near 37° C. immediately after milking retards the rate of subsequent lipolysis upon cooling (Curve IV). In another experiment, when milk was held for two and one-half hours at 33° C., the development of perceptible rancidity upon cooling did not take place until after 30 hours of storage; whereas, a strongly rancid flavor developed after 12 hours in a sample of the same milk that was cooled immediately after milking.

The role of cooling in the mechanism of spontaneous lipolysis became evident in the study of lipolytic activity in various milk fractions obtained from cooled and uncooled milk and, particularly, from washed creams.

The procedure for washing creams was as follows: Separated cream of 25 to 27% fat was diluted with four parts distilled water and reseparated. The process was repeated twice. Final washing was done with a phosphate-citrate buffer of pH 6.65. (This is essential because lipolysis is limited by a rapid drop of pH if washed cream is not buffered.) All cream separations and washings were carried out at 36 to 38° C. Distilled water was brought up to 38° C. before adding it to cream. Experimental work with milk began within 15 to 30 minutes after milking. When necessary, milk in transit from milking barn to laboratory was maintained at from 35 to 38° C.

Typical data on the lipolytic activity of washed creams obtained from naturally active milk and normal milk, both precooled and not precooled, are shown

(Figure 2). In this and other experiments, the washed cream from milk not precooled was obtained by the separation of freshly drawn milk maintained at from 35 to 38° C., followed by washing of the cream. For precooled milk experiments, milk was rapidly cooled to 5° C., held for one hour (½ hour to two hours

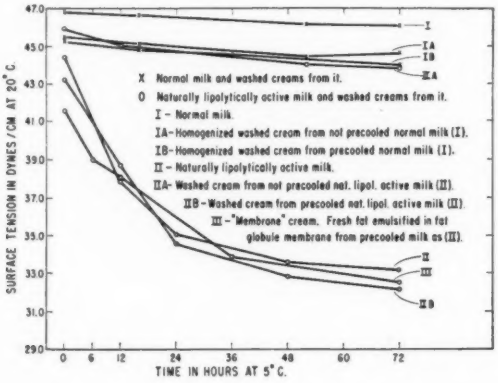
SURFACE TENSION IN DYNES/CM AT 20° C.
TIME IN HOURS AT 5° C.
X Normal milk and washed creams from it.
O Naturally lipolytically active milk and washed creams from it.
I – Normal milk.
IA – Homogenized washed cream from not precooled normal milk (I).
IB – Homogenized washed cream from precooled normal milk (I).
II – Naturally lipolytically active milk.
IIA – Washed cream from not precooled nat. lipol. active milk (II).
IIB – Washed cream from precooled nat. lipol. active milk (II).
III – "Membrane" cream. Fresh fat emulsified in fat globule membrane from precooled milk as (III).

FIG. 2. Lipolytic activity of washed creams.

in other experiments), and warmed to 37° C., for separation and washing of the cream. Prepared samples were cooled and aged in cold. The zero-time surface-tension values were taken immediately following cooling.

Data show (Curves II, II B, III) that in the case of precooled, naturally active milk, the lipolytic activity of the creams freed from milk plasma by washing was essentially the same as, or greater than, that of cooled original milk. Washed cream from the same milk not precooled (Curve II A) was substantially free of lipolytic activity. It is evident that a naturally active lipase is in the plasma of milk before cooling. By cooling the milk, the lipase is adsorbed on the fat globules, i. e. substrate, and lipolysis proceeds. If milk is not cooled, the adsorption is not effected and there is essentially no lipolysis (Curve II A). Tested organoleptically, this cream was not rancid after 72 hours of aging, while the cream from precooled milk (Curve II B) was strongly rancid when tested after two hours of aging. The extensive hydrolysis of fresh milk fat emulsified in the membrane material isolated from precooled milk (Curve III) confirms that the enzyme has been adsorbed on the fat-globule surface by cooling. Isolation of the fat-globule membrane material from precooled, naturally active milk ("membrane cream," Curve III) can serve as the source and starting preparation for further purification of this lipase. The acid degrees of fat of "membrane cream" before emulsification and after emulsification and aging for 72 hours were, respectively, 0.26 and 12.67. Adsorption of naturally active lipase by cooling is irreversible. Washed creams, from precooled milk, which were rewarmed and aged at 37° C. exhibited an even higher rate of lipolysis.

Cooling of normal milk had no effect on the partition of the lipase of normal milk. The lipase remains in the plasma; therefore, in the corresponding washed creams (Curves IA, IB), even though homogenized, there was no lipolysis.

Partition of lipolytic activity in normal milk. Studies in Part I dealt with naturally active milk of high lipolytic activity. Precooling treatments were necessarily brief (½ hour to two hours), although they were ample to demonstrate adsorption of naturally active lipase. If naturally active lipase is present only in small concentrations, as it may be in normal milk *(28)*, longer cooling treatments might be necessary to demonstrate the presence of this lipase by adsorption on fat-globule membrane. In addition, determination of lipolytic activity of the membrane toward simple esters as substrates would throw light on partition of the esterases of milk.

Freshly drawn, mixed normal milk that had never been cooled was divided into two portions. One portion was processed immediately; the other was aged at from 0 to 5° C. for 24 hours. The first portion was separated and the resulting skimmilk was frozen and lyophilized. The cream was washed with distilled water, the washed cream churned, and the isolated membrane material lyophilized. Lyophilized membrane material was prepared similarly from the portion that was aged. The lypohilized materials were reconstituted in distilled water with the Omni-mixer just before activity determinations.

Results of this experiment are presented (Table 1). It is evident that the lipolytic activity of the membrane material is increased appreciably after cooling the milk from which it is derived and aging it in the cold for 24 hours. This adsorption of lipase activity in the membrane material of normal milk after cooling for 24 hours would indicate the presence of naturally active lipase in this milk.

TABLE 1

Effect of aging normal milk at a low temperature on the relative lipolytic activity of its skimmilk and membrane material

Milk history	Milk fractions	Substrates [a]	Activity	Relative activity
			(units/10 g. S. N. F.[b])	
Fresh, noncooled	Skimmilk	T-20	3.68	1.2
		MB	2.44	0.80
		TB	8.43	2.8
		MF	3.05	1.0
Cooled and aged 24 hrs. at 0 – 5° C.	Skimmilk	T-20	3.08	1.4
		MB	2.19	1.0
		TB	8.82	4.1
		MF	2.14	1.0
Fresh, noncooled	Membrane material	T-20	0.42	0.70
		MB	0.12	0.20
		TB	1.40	2.4
		MF	0.59	1.0
Cooled and aged 24 hrs. at 0 – 5° C.	Membrane material	T-20	8.22	1.5
		MB	2.67	0.50
		TB	17.3	3.2
		MF	5.34	1.0

[a] Abbreviations used throughout the paper: T-20 = Tween-20; MB = methyl butyrate; TB = tributyrin; MF = milk fat.

[b] S. N. F. = Solids-not-fat.

but in small concentrations. The difference in the relative activity of the membrane material from cooled and aged milk and the skimmilk from the corresponding milk could be attributed to a preferential adsorption of the enzyme system. However, this is complicated by a simultaneous differential loss in stability of the enzyme system by the aging process *(7)*.

It was possible to use shorter aging periods with naturally active milk of slight lipolytic activity,[5] thus minimizing any loss of stability of the enzymes. This milk was aged four hours at 0° C. immediately after milking, and the skimmilk and corresponding membrane materials were prepared as described above. The lyophilized materials were stored below 0° C. until assayed.

Data for the relative lipolytic activity of the skimmilk and membrane material from this milk are presented (Table 2). Preferential adsorption is indicated

TABLE 2

Lipolytic activity of skimmilk and membrane material fractions obtained from slightly naturally active milk[a]

Milk fractions	Substrates	Activity	Relative activity	Specific activity	Enrichment
		(units/10 g. S. N. F.)		*(units/g protein N)*	
Skimmilk	T-20	2.71	1.5	0.70	.....
	MB	1.31	0.73	0.34	.....
	TB	11.2	6.2	2.87	.....
	MF	1.80	1.0	0.46	.....
Membrane material[b]	T-20	48.3	1.2	7.43	11
	MB	16.6	0.43	2.55	7.6
	TB	226	5.8	34.7	12
	MF	38.9	1.0	5.98	13

[a] After aging at 0° C. for four hours.

[b] Analysis of lyophilized material: 60% fat, 31% protein, 6% moisture.

from the specificity and enrichment data. The relative lipolytic activity with respect to methyl butyrate is lower in the membrane material than in the corresponding skimmilk. Therefore, the enzyme system of the membrane material seems closer to a true lipase than that of the skimmilk. This is in harmony with the greater heat stability reported for the lipase activity in naturally active milk *(24)*.

The skimmilk and membrane material obtained from 800 gm. of the milk produced a total activity (toward milk fat) of 124 and five lipase units, respectively. Therefore, although an enrichment of 13 times was obtained in the lipase activity in the membrane material, the total activity of this fraction represents only about 4% of the total lipolytic activity of this milk. In normal milk this portion was less than 1%. It would have been interesting to obtain similar data on the milk of high natural lipolytic activity, but such milk was not available at the time this work was carried out.

[5] On aging for 24 hours at 5° C. the surface tension of this milk was lowered to 43.0 dynes/cm. Organoleptically, it was scored only slightly rancid.

In Part I it was shown that spontaneous lipolysis is greatly inhibited if the milk is kept at 37° C. for from one to three hours before cooling and aging at 5° C. The effect of this treatment was studied on the relative adsorption of the lipase system of the membrane material in normal milk. For this experiment, freshly drawn mixed milk was divided into two fractions. One was cooled immediately and aged at from 0 to 5° C. for 24 hours; the other was first kept at 37° C. for three hours and then cooled and aged for 24 hours. After aging, the corresponding skimmilk and membrane material from each fraction were prepared and lyophilized for the assays. The results (Table 3) show that the inactivation

TABLE 3

Effect of holding milk at 37° C. for three hours prior to cooling and aging at a low temperature

Treatment of milk	Substrates	Skimmilk activity inactivation		Membrane material activity inactivation	
		(units/10 g. S. N. F.)	%	*(units/10 g. S. N. F.)*	%
Cooled immediately and aged for 24 hrs. at 0 to 5° C.	T-20	3.14	.....	9.28	.....
	MB	1.56	.....	7.24	.....
	TB	8.26	.....	36.4	.....
	MF	1.61	.....	10.4	.....
Kept at 37° C. for three hrs., then aged at 0 to 5° C. for 24 hrs.	T-20	1.74	45	2.41	74
	MB	1.12	28	3.85	47
	TB	7.27	12	23.6	35
	MF	1.54	4.3	4.23	59

resulting from this treatment is much greater for the activity of the membrane material than for the activity of the skimmilk. This difference could be attributed again to different enzyme systems involved in the plasma and in the membrane material, the latter being more sensitive to this treatment. An alternative interpretation of these results is that keeping the milk at 37° C. prior to aging in the cold may impair the adsorption of the enzyme system or its ability to be adsorbed on the membrane material.

The pH-activity curve was determined for the activity of the membrane material (toward milk fat) obtained from milk of slight natural lipolytic activity. Two distinct pH optima were obtained, one at pH 8.8 and a smaller one at pH 7.5. It is difficult to interpret these pH activity peaks in terms of multiple enzymes, because of the numerous factors that influence the shape of the pH-activity curve of an enzyme *(6)*.

Casein was prepared from fresh mixed normal milk by centrifugal sedimentation and rennet coagulation. Acid precipitation was not used for this purpose, because of the marked inactivation of milk lipase at pH levels below 6.6 *(7, 8)*.

The Sharples supercentrifuge was used to obtain large quantities of casein that could be lyophilized or used as such. For the specificity experiments a batch procedure was used by passing 250 ml. of skimmilk into the Sharples hollow bowl running at 56,000 r.p.m. and centrifuging for 10 minutes at room temperature. The residual casein nitrogen of the whey in one run was 6% of the total casein

nitrogen in the skimmilk. The casein gel was collected from the inside of the centrifuge bowl. A 10% suspension was prepared by redispersing the casein in distilled water with the Omni-mixer at low speed, to avoid stable foam formation.

The relative lipolytic activity of the casein gel was compared with that of the original skimmilk. From the specificity data (presented in Table 4) it appears

TABLE 4

Relative lipolytic activity of skimmilk and casein

Enzyme sources	Substrates	Activity	Relative activity
		(units per 100 ml.)	
Skimmilk	T-20	3.02	1.2
	MB	2.84	1.1
	TB	14.2	5.4
	MF	2.61	1.0
Casein (10%)	T-20	2.21	0.90
	MB	2.36	0.90
	TB	8.88	3.5
	MF	2.51	1.0

that the lipolytic activity of the casein toward all simple esters, especially tributyrin, decreases in relation to the activity toward milk fat. The fact that the relative lipolytic activity of milk does not remain constant upon fractionation is considered to be additional evidence for the presence of multiple lipolytic enzymes in milk.

The casein gel obtained with the Sharples centrifuge was compared with rennet casein. Renet coagulation of skimmilk was carried out as follows: Fresh skimmilk was measured into 60-ml. centrifuge tubes and adjusted to 40° C. in a water bath. A 1% rennin solution was added at the rate of 0.5-ml. per 40 ml. of milk, mixing thoroughly. A firm clot was obtained in 10 minutes. The curd was cut into sections with a sharp knife and then allowed to stand for one hour at room temperature. The tubes were then centrifuged at low speed, and the whey was poured off. The sedimented clot was resuspended in distilled water, as described above.

Results (Table 5) show that although lipase in the casein fractions could not be enriched, the recovery obtained was good (by the two procedures). These casein preparations would constitute suitable starting material for purification work with milk lipase. It is evident that the major portion of lipase in normal milk is associated with the casein fraction. This has been accepted in the literature, but no data have been published heretofore on the relative concentration of lipase in this fraction. This study indicates that the lipase activity of casein constitutes 80 or 90% of the total activity (that of casein plus whey) in a given amount of milk.

To determine whether milk lipase was associated with any size of aggregate of the casein complex, skimmilk was subjected to differential sedimentation with a Servall SS-1 supercentrifuge. Sedimentation was carried out in a cold room

TABLE 5
Lipase activity[a] of skimmilk fractions

Treatment	Fractions obtained	Volume	Activity	Total activity	Protein N	Specific activity	Yield
		(ml.)	*(units/ml)*	*(units)*	*(mg/ml)*	*(units/g (protein N)*	*(%)*
Sharples	Skimmilk	250	0.0322	7.5	35.3	0.91	100
centrifuge,	Casein gel	(260)[b]	0.0205	5.3	25.1	0.82	71
56,000 r.p.m.,	Whey	220	0.0060	1.3	9.7	0.62	17
10 min.	Skimmilk	80	0.0335	2.7	34.0	0.99	100
Rennet	Casein curd	(100)[b]	0.0251	2.5	22.1	1.14	94
coagulation	Whey	57	0.0046	0.26	8.1	0.57	10

[a] Substrate: Milk fat.
[b] Made-up volumes.

at 5° C. for various lengths of time up to four hours at full speed (25,000 g.). The activity of the fractions obtained at various centrifuging times showed that lipase was not associated preferentially with any particular size of casein aggregate. Poor recovery of lipase in the casein fractions probably was obtained by this procedure because of prolonged centrifugation.

DISCUSSION

Data on the partition of the lipolytic activity of milk between plasma and fat-globule membrane, and on the relative lipolytic activity of these fractions toward various substrates show that, besides simple esterases, milk contains two distinct lipases. One is "the lipase of normal milk," which is associated with the caseinate fraction, and the other is "naturally active lipase," which is preferentially and irreversibly adsorbed onto the fat-globule membrane material when milk is cooled. Both enzymes are present in all milks; but in certain milk — so-called "naturally active milk," usually from cows late in lactation and on dry feed — the naturally active lipase is present in high concentration. Concentration of this lipase varies from a very low one in normal milk, which will not develop perceptible hydrolytic rancidity on cooling and aging for over 48 hours, to milk of high natural lipolytic activity, which will become strongly rancid within one-half hour after cooling. Factors other than enzyme concentration, such as the possible presence of inhibitor *(3, 23)*, may affect the activity of naturally active lipase and therefore may play a role in determining the degree of spontaneous lipolysis in various milks. However, the possibility that one and the same lipase is involved in both "induced" and "spontaneous" lipolysis is ruled out by the great difference in concentration of the naturally active lipase in spontaneous milk and its concentration in normal milk; by the preferential adsorption of naturally active lipase onto the membrane material upon cooling of milk, and by the difference in the relative lipolytic activity of membrane material and the corresponding skimmilk toward different esters. It is remotely possible that the preferential adsorption might be attributed to the difference in adsorption characteristics of the fat-globule membrane in different milks, rather than to the distinct lipase systems; however, the evidence on the difference in the relative lipolytic activity toward different esters makes such an explanation untenable.

The modes of lipolysis produced by lipase of normal milk and by naturally active lipase are different. In freshly drawn milk, both lipases are in the plasma of milk. Upon cooling of milk, the adsorption of naturally active lipase on the fat-globule surface takes place and the lipolysis is effected. On the other hand, the lipase of normal milk remains in the plasma and 90% of its total activity is associated with the caseinate fraction. To bring about lipolysis by the lipase of normal milk, certain "activation treatments" (homogenization, temperature treatments, or agitation) are necessary. It has been commonly regarded that the entire effect of these activation treatments is on the substrate *(9)*. It was postulated *(26)* that the natural membrane material around fat globules as it exists prior to activation protects the fat from lipolysis. Recent evidence on the mechanism of lipolysis by air agitation *(22)* indicates that the activating treatment is dependent for its effectiveness not only on changes in the substrate but also upon effects on the enzyme itself. The lipase of normal milk is in the caseinate fraction. The fat-globule membrane material is free of casein. To effect or to induce the lipolysis in normal milk, the caseinate fraction carrying lipase must be preferentially concentrated in the fat-plasma interface and become a part of the adsorption layer around the fat globules, for the union of enzyme with the substrate to take place. The role of "activating treatments" is to promote and accomplish the adsorption of the enzyme on the surface of fat globules where lipolysis occurs. Adsorption of naturally active lipase is accomplished simply by cooling the milk. Lipase of normal milk remains in the plasma after cooling; therefore, "activating treatments" are needed to bring about adsorption of the enzyme upon the substrate. Such changes in the natural adsorption layer around the fat globules as accomplished by activation treatments are necessary for adsorption of the lipase of normal milk on the surface of fat globules. In this sense, the natural membrane around the fat globules does protect the fat from lipolysis. Once the enzyme is adsorbed on the fat-globule surface, it is doubtful that the membrane material surrounding the fat globules could protect the fat from lipolysis. The natural adsorption layer around fat globules is neither continuous nor absolutely impervious *(1)*, especially to such relatively small protein molecules as the enzymes. This is certainly true in lipolysis by a naturally active lipase or by the addition of steapsin to milk *(26)*, in which it is not necessary to "resurface" or "denude" fat globules for lipase action on fat as it exists in milk.

The fact that both milk lipases are in the plasma of freshly drawn milk, and that their respective adsorption on the substrate requires cooling in one case and activation treatment in the other, explains why milk as it comes from the cow is never rancid.

Distinction between the two lipases is of great importance in the control of rancidity in milk. Because of their differences in mode of lipolysis, the control measures are necessarily different. Failure to distinguish between the mode of action of these two lipases and to agree on their terminology has further complicated an understanding of rancidity and its control. The authors propose

to designate the naturally active lipase — that adsorbed on the fat-globule membrane material upon cooling of milk — as the "membrane lipase" of milk; and they propose to designate the lipase of normal milk — that involved in induced lipolysis — as the "plasma lipase" of milk.

CONCLUSIONS

All cow's milk contains at least two different lipase systems. One is "naturally active lipase," which is irreversibly adsorbed on the fat-globule membrane material upon the cooling of freshly drawn milk; the other is "lipase of normal milk," which remains in the plasma and is associated with the caseinate fraction.

The difference between naturally lipolytically active milk and normal milk is the difference in concentration of naturally active lipase. Naturally active milk, which usually comes from cows late in lactation and on dry feed, contains the naturally active lipase in high concentration.

The naturally active lipase can be separated from the lipase of normal milk by washing (freeing from milk plasma) the cream obtained from precooled, freshly drawn milk and isolating the membrane material upon which the enzyme is adsorbed.

Data on partition and specificity of two lipase systems have been presented, and the mode of lipolysis by each enzyme has been discussed.

To distinguish clearly the relative group specificity of these two lipase systems and their different modes of lipolysis, it is proposed that the naturally active lipase (that adsorbed on fat-globule membrane material on cooling of milk) shall be called the "membrane lipase" of milk, and that the other be called the "plasma lipase" of milk.

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OUR INDUSTRY TODAY

Brief Reviews of Current Topics

Reducing Steam Costs in the Dairy Plant

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Each year over five billion pounds of milk are processed in Ohio (and Michigan). This requires one billion pounds of steam at a cost of three million dollars, if carried out according to present practices. When using faulty equipment and following poor practices, the cost of steam is often actually 20 per cent greater than it should be.

Steam Needs

The major steam requirements are as follows:

1) Can-washer—three pounds of steam per 100 lb. of milk.
2) HTST pasteurizer—seven pounds of steam per 100 lb. of milk.
3) Bottle- and case-washer—five pounds of steam per 100 lb. of milk.
4) Clean-up—5 lb. of steam per 100 lb. of milk.

This makes a total of approximately 20 lb. of steam for 100 lb. of milk processed, at a cost of $0.06.

Cost

Steam costs should be analyzed on the basis of:

1) Pressure used.
2) Boiler efficiency.
3) Piping and valve operation.
4) Utilization of steam at processing equipment.

The cost of steam in a medium-small plant varies considerably, from $2.50 to $6 for each 1,000 lb. Cost of steam for a particular plant does not vary greatly with different sizes of boilers (Fig. 1). For example, a plant processing 75,000 lb. of milk per day would have a steam cost of $2.30 for a 75 BHP boiler, as compared to $2.50 for a 150 BHP boiler. The cost of steam depends primarily upon labor costs, and in many areas an extra man is required for operating a boiler for generating high steam (above 15 p.s.i.). By operating the boiler at low pressure (below 15 p.s.i.) the cost of steam would be reduced one-half; that is, instead of $3 per 1,000 lb. of steam, the cost would be $1.50.

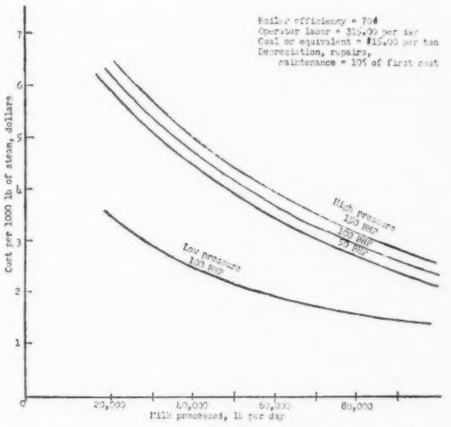

FIG. 1. Estimated steam costs for dairy plant using low and high pressure.

Low-Pressure Steam

You might ask, "Why has high-pressure steam been used in the dairy plant in the past?" The principal reason is that the can-washer is often supplied with high-pressure steam at a temperature of 212 degrees Fahrenheit and higher for the final rinse. In order to provide high-temperature steam at the can-washer, pressures up to 75 lb. per sq. in. are generated at the boiler. Also, some pasteurizing systems require high-pressure steam. With the elimination of the receiving rooms of many dairy plants, the need for high-pressure steam is reduced. The installation should be made so that it can be used for high pressure steam if needed at a later date. With well-insulated steam lines, and steam at ten p.s.i. supplied to the equipment, a temperature of

Presented at the Ohio Dairy Technology Conference, Columbus, Ohio, February 5-8, 1957.

240 degrees Fahrenheit will prevail. If a low-pressure steam system is used

1) A special boiler operator would not be required in most areas.
2) Steam at 10 p.s.i. and 240 degrees Fahrenheit can be delivered to the equipment if steam lines are insulated.
3) A larger-sized pipe is needed, e.g., to deliver 55 lb. per min., a 4-in. diameter pipe is needed for steam at 10 to 15 p.s.i.; whereas, a 3-in. pipe is needed at 75 p.s.i.
4) Maintenance problems will decrease. Traps, including the operation and controls, give less trouble.
5) Fuel requirements decrease.

Efficiency and Piping

The possibility of reducing costs in steam operations is unlimited. The following savings can be obtained in practically every plant:

1. By increasing the efficiency of the boiler five per cent, it is very easy to make a savings of one-half pound of steam per 100 lb. of milk processed. Boiler efficiency is improved by:
   (a) Keeping the transfer tubes clean on water and heated side.
   (b) Maintaining proper fuel combustion.
   (c) Maintaining proper air-fuel ratio.
   (d) Selecting fuel wisely.
2. By providing insulation on 50 ft. of 3-in. steam line in a medium-sized plant a savings of one-fifth pound of steam per 100 lb. of milk is made.
3. Steam leaks around valves and connections cause considerable loss. This loss is from zero to one-half pound of steam per 100 lb. of milk.
4. By returning the condensate to the boiler, the reduction in steam cost, in addition to reducing problems of water treatment, provides a savings of three-fourths pound of steam per 100 lb. of milk.

These provide a possible savings of one and one-half to two pounds of steam per 100 lb. of milk for miscellaneous losses, over and above the savings which can be made by the efficient use of steam at the equipment. A savings of two pounds per 100 lb. of milk would provide a daily savings of $2.40 in a plant handling 40,000 lb. per day.

Equipment

It is possible in many operations to make the following savings in steam requirements at the equipment:

1) Can-washer—one pound of steam per 100 lb. of milk.
2) HTST pasteurizer instead of a batch—five pounds of steam per 100 lb. of milk.
3) Bottle- and case-washer—one pound of steam per 100 lb. of milk.
4) Clean-up—one pound of steam per 100 lb. of milk.

These provide a potential savings of eight pounds of steam per 100 lb. of milk.

Thus, on a 40,000 lb.-per-day processing operation, 2,000 lb. of steam, or $6 per day, could be saved by using the short-time instead of the batch-pasteurizing operation. Many of you have already made that change. The savings at the can-washer, bottle- and case-washer, and clean-up provide an additional potential savings of 1,200 lb. of steam, or $3.60 per day.

How can these savings be accomplished? The following check list can be used to determine ways to effect savings:

1) Controls or valve handles on steam lines should be readily accessible to the operator.
2) Valves and jets in the washer should work properly.
3) Proper kinds and amounts of washing compounds for wash water for can-washer and bottle- and case-washer should be used.
4) Heat-transfer surfaces should be kept clean.
5) Regenerative heating-cooling should be used for conserving heat.
6) Condensate should be returned to the boiler wherever possible.
7) Excessive rinsing of equipment with hot water while cleaning should be avoided.
8) Excessive overflow from bottle- and case-washer should be avoided.

Summary

The possibility of increasing efficiency, decreasing the steam requirements, decreasing the unit costs, and thereby the total cost of steam operations for your plant is in your hands. Start at the boiler. Analyze your needs with respect to pressure, and possibly eliminate high-pressure steam systems. With low pressure, the cost of steam can be reduced one-half for plants processing less than 80,000 lb. per day. For instance, a 40,000 lb.-per-day plant spends approximately $24 per day for high-pressure steam, but by using low-pressure steam, the daily cost of operation can be reduced to $12 or less per day. Then, analyze the efficiency of the boiler and its distribution system, including valves, connections, insulation, etc. Trace the steam to the equipment in which it is used. Check the operation of equipment, to make sure that every pound of steam which enters the equipment does useful work. By using better methods each plant could on the average reduce its steam requirements by five pounds for each 100 lb. of milk processed. This would amount to a daily savings of approximately $6 per day for a plant handling 40,000 lb. per day.

Reducing Refrigeration Costs in the Dairy Plant

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Each year over five billion pounds of milk are processed in Ohio (and Michigan). This requires an estimated fifty million ton-hour of refrigeration, and at $0.06 per ton-hour, an annual cost of three million dollars. The cost of refrigeration for a 30,000 to 70,000 pound-per-day plant, based on today's equipment prices, is about $0.06 per ton of refrigeration for each hour. Some plants are getting their refrigeration at a much lower cost, because their installations were made when lower prices prevailed. One ton of refrigeration will remove 12,000 B.t.u.'s per hour.

To analyze refrigeration costs, (1) check operation efficiency, (2) check refrigeration needs and use at equipment.

Refrigeration Needs

The major refrigeration requirements for a 40,000 pound-per-day plant are as follows:

(1) Cooling raw milk through 20 degrees F. —70 ton-hours, $4.20.
(2) High-temperature short-time pasteurization—98.7 ton-hours, $5.92.
(3) Refrigerated storage for milk—216 ton-hours, $13.

This makes a total of $23.12.

How can these refrigeration costs be reduced? The main operating cost is the electricity, which amounts to about 1 kw.-hr. per ton, with the major usage of electricity in the plant being for the refrigeration compressor.

Reducing Costs

The major operating technique which should be understood by every plant manager and by the operator of the equipment is that the head pressure should be kept as low as possible. When operating at 150 p.s.i. head pressure instead of 200 p.s.i., the operational cost is reduced 16.5 per cent, for a daily cost of $3.20. The head pressure is affected and controlled mainly by the amount and temperature of cooling water supplied, and by the cleanliness and area of condenser surfaces. It may be economical in many plants to reduce the cost of cooling the condenser by use of a cooling tower to decrease the fresh-water requirements. It is often possible to pay for a cooling tower in five years by the savings in water costs, depending on the cost of the water being used. Another important factor in operating the compressor is that the suction pressure should be kept as high as possible. By operating at 35 p.s.i. instead of 25 p.s.i., the capacity of the system is increased 30 per cent. On the example above, this would amount to a reduction in daily operating cost of $2.30 (10 per cent of operating costs). Another method of reducing refrigeration costs, particularly for cold storages, is to provide a method of defrosting such that the refrigeration load from the storage is not lost.

Use of ice builders will help keep demand low, with consequent low electrical charge. Keep oil out of the refrigeration system. Keep noncondensable gases from the system. As with heating, the cooling surfaces should be kept clean. Performance of the system should be checked regularly. The quickest and easiest way to save on refrigeration costs is to minimize the needs for refrigeration, through use of improved processing methods, such as cold separation and high-temperature short-time pasteurization. Cold water, where available, should be used for all cooling.

Partial Check List for Refrigeration Operation

1. Maintain high suction pressures (as high a temperature as possible in evaporator).
2. Maintain low head pressure.
3. Check expansion valve monthly (frost should not occur on outside of pipes back to compressor).
4. Purge system of oil.
5. Remove noncondensable gases.
6. Keep condenser tubes clean.
7. Defrost evaporator regularly.
8. Use two-stage systems for low-temperature applications.
9. Control system automatically.
10. Consider use of cooling tower, if water is purchased or of low supply.
11. Select oil which will not solidify or evaporate.
12. Keep compressor in good condition—valves, pistons, rings, bearings.
13. Check entire system for leaks.
14. Check motor, condition of belt, belt tension, alignment of motor and belt.
15. Check losses of refrigerated air or product.

Summary

The first step in reducing costs of refrigeration is to produce the refrigeration efficiently, by operating at as high a suction pressure and at as low a head pressure as possible, while still maintaining the desired evaporator temperature. By following these practices, it is estimated many plant operators can reduce their costs on an average of $3 per day for a plant

Presented at the Ohio Dairy Technology Conference, Columbus, Ohio, February 5-8, 1957.

with 40,000-lb. capacity. The next step is to reduce the need for these refrigeration services wherever possible. Then study each individual operation, to locate sources of energy losses. The principles are the same, whether steam or refrigeration are involved. If the plant operations are observed critically, costs can be reduced and income increased.

Recent Developments in Refrigerated Transportation[1]

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During the last few years there have been many changes in refrigerated transportation equipment, including types of bodies and methods of refrigerating them. More rigid health requirements for delivering products to dealers and to consumers have made it necessary to depart from standards formerly considered acceptable.

Kinds of roads, sizes of routes, whether they are in heavily populated, urban centers or in sparsely settled, rural areas, where distances between cities are greater, have resulted in the development of different kinds of transportation equipment and in methods of refrigerating them to meet these requirements. Those of us who are responsible for the activities of our companies have the knowledge for only general supervision. We rely on the specialists in our organization for the detailed and technical knowledge required to properly take care of the various functions involved in the production of refrigerated bodies.

Primarily, bodies used for delivery of perishable food products should be kept sanitary. Such bodies should be lined with metal sheets, so they can be thoroughly and frequently washed, without moisture entering the insulation or the structural members, causing deterioration and impairing efficiency. Floors of bodies should be protected by a metal floor pan, extending up the side walls a reasonable distance. Thickness and type of floor pan depend entirely on type of load to be carried. Doors should be low and close enough to the ground to facilitate easy access to the products, and kick plates should be installed under each door opening; otherwise, the paint will soon become scuffed and the protection it affords will be lost. Doors should be equipped with safety releases. Cut-out steps should be provided under each door, to facilitate entry, or ease of reaching into the body. (Lack of this feature in many instances has been the cause of serious injury to the truck operator.) The number and variety of articles contained in the average load in an ice cream delivery body, for example, have emphasized this matter of easy access, and have resulted in the use of doors on each side of the body—considered very dangerous and unnecessary years ago, but now generally accepted without question.

Provision must be made to use rust-resistant material or inhibitors in body construction, and to prevent and combat entry of moisture into the insulation and structural members. In low-temperature truck bodies a properly and efficiently designed vapor seal will greatly assist in keeping moisture out of insulation. Also, an ounce of prevention, so-called, will produce more than a pound of cure, if processors will desist from the popular practice of defrosting a low-temperature hold-over plate by means of a hose, using either cold or hot water. This practice is the cause of many moisture problems found in insulated and refrigerated bodies. Refrigeration requirements should be carefully calculated and based on size of body, thickness of insulation, time away from plant, number of times doors are opened, and the worst possible climatic conditions. It is much better to over-refrigerate a unit than to lose a payload because the job was equipped with just enough refrigeration to handle average conditions.

Briefly, these are some of the items which all manufacturers of good refrigerated truck bodies check carefully in producing units for your use. There are now in use several different styles of bodies and methods of refrigeration. Some of these are: *(1)* The commonly accepted hold-over plates in which the eutectic solution is frozen with either a connection with a central ammonia system at the plant or a self-contained Freon condensing unit carried on the truck body. *(2)* A continuous over-the-road system[2] with a stand-by condensing unit for use when the body is being loaded or is back at the main plant. This system has become

[1] Presented at the Twenty-fourth Annual Dairy Technology Conference, The Ohio State University, Columbus, February 7, 1957.

[2] Some continuous refrigeration systems: THERMO KING—blower; COLDMOBILE—blower with stand-by; MARK B, by Tranter—blower with stand-by; CROWN, by Tranter—hydraulic with stand-by; GENERAL ELECTRIC—electric with stand-by; ONAN GENERATOR—gas engine with electric stand-by; ROVAN, by G. E.—electric blower with stand-by; BATAVIA—hydraulic with stand-by.

more widely used, because of larger body capacity, longer routes—which necessitate absence from the manufacturing plant for one or more days—and reduction in number of manufacturing bases as sources of supply for the delivery equipment. These over-the-road systems are available for use not only on large wholesale units but are also adaptable to semi-trailer bodies, on which they may be connected to a motorized axle which furnishes the power while the unit is traveling over the road and makes the trailer body itself independent of any one tractor. This type of installation also has the stand-by unit for use wherever it may be based overnight.

(3) There are the blower systems for use in either high- or low-temperature work, operated by a gasoline motor, by a drive-shaft connection, or by being belted directly to the truck engine. All of these systems have their places, based on requirements of individual users. *(4)* Large-capacity bodies for wholesale delivery of ice cream or other dairy products have become essential. The use of the small-capacity unit is past. Capacities of ice cream bodies now average between 1,000 and 1,600 gallons or more, for regular wholesale routes. *(5)* Use of semi-trailers for delivery of ice cream from the manufacturing base to distant distributing points is definitely increasing. The capacity of these trailers will average almost 5,000 gallons. *(6)* Wholesale milk or dairy products other than ice cream units will now have an average carrying capacity between 175 and 275 cases of milk, and almost all are refrigerated. Delivery of dairy products or other perishable foods to the consumer in a non-refrigerated body is rapidly becoming a thing of the past. Not only is this true of wholesale delivery but it is also becoming more and more in effect for retail or house-to-house delivery. *(7)* More rigid restrictions by various state laws, on the larger capacity equipment, as to permissible weights on each axle, have resulted in body manufacturers devising ways and means of reducing the empty weight of the bodies and the refrigeration systems. This is being done by using lighter-weight metals, lighter-weight insulating materials, and by installing thinner hold-over plates in the case of over-the-road refrigeration systems. Several hundred pounds of hold-over plates alone may be saved by using $\frac{7}{8}$-inch instead of $2\frac{5}{8}$-inch plates in large-capacity bodies where a continuous refrigerating system is practicable. *(8)* At present, there is a trend toward the use of aluminum hold-over plates, instead of the steel types which have been commonly accepted for years. Claims are made for these aluminum plates that, in addition to the weight saving, they *(a)* have a greater rate of heat transfer, *(b)* contain more feet of refrigerant tubing, *(c)* have a greater B.T.U. hold-over capacity, *(d)* eliminate or greatly reduce the frost build-up on the plates, etc.

Body manufacturers are continually experimenting with aluminum, magnesium, and more recently, plastic, for use in various parts of the bodies, not only for cutting down the weight but for securing a better deterrent of moisture-conduction into the insulation and structural members.

Aluminum sheets are being cast for experimental use as exterior panels, with lengthwise openings which carry the refrigerant. This would be practically a direct expansion system, with no hold-over feature, and would be for constant or continuous refrigeration systems only. Some of the outstanding features claimed for plastic panels reinforced with Fiberglas are, that they *(a)* have a high resistance to rust and corrosion, *(b)* have excellent insulating qualities, *(c)* effect a considerable reduction in body weight, *(d)* greatly improve the body sanitation, *(e)* reduce maintenance, and are easy to repair, because they are resilient and not easily dented. Also, this material is not an electrical conductor, and apparently will not absorb odors. It may be easily painted any color.

It is believed that you may look for rapid advances along these lines in the next few years. *(9)* A very important factor for processors is the time consumed in loading the bodies, and in delivering the daily load. Doors on both sides of ice cream bodies have already been referred to; they have been definitely accepted as a great time-saver on wholesale routes. During the last couple of years, another style of body has come into use for wholesale ice cream delivery, which it is claimed saves as much as two hours a day on routes averaging 40 customers. This is what is commonly called a walk-in type body, with entry through almost a full-height rear door.

Arriving at any delivery point, the driver enters the body through this large rear door, which is equipped with an inside safety latch. Unless the driver is well over average height, he works easily, without stooping, as he moves up and down the wide aisle which is in the center of the body. He quickly selects the items he wants from the stock, on the shelves that extend almost the entire length of the body on each side.

Items picked for each delivery are placed to the right rear side of the body, adjacent to a curb-side door. When the order is ready to take into the store, the driver, emerging from the truck, goes around to the curb side for just a moment, takes a hand-truck from the back of the body and, setting it in place, stacks the order and makes rapid delivery. With only two doors to open instead of as many as six, the driver presumably saves time and footwork. *(10)* Another method of saving time has been adapted to semi-trailers, equipped with conveyors which speed up the loading and permit the body to get to the destination sooner, also expedite the time when the unit may be

connected to the refrigerating system after the loading operation is completed. *(11)* For wholesale-milk bodies, a new system of loading has been introduced, called pallet-loading. A specially designed body which permits high-speed pallet-loading is equipped with full-width rear doors which, when open, expose the entire rear loading area and make the full width of the body accessible. The floor inside the body has been raised above the wheel housings, to the height of the normal loading dock. Special raised bumpers afford quick spotting of the truck at the docks and permit the use of dock boards without danger of damage to the body.

An extruded aluminum floor provides light-weight, rust-proof, nonslip surface. It is pitched to drain quickly, is easy to flush out, and replaces the corrugated steel floors in other type models. Hold-over plates are located high around the inside perimeter of the walls, to allow loading the pallets, stacked five cases high, flush with either side or front wall. All refrigeration lines are recessed within the walls to eliminate danger of breakage. Users of this type of body, equipped with an automatic lift-cart, pick up 45 cases of milk on a pallet, deposit the payload, and return to the warehouse to repeat the process seven times. This special body has a capacity of eight pallet loads (7 full pallets and 1 empty), and is loaded by only a single worker.

Deliveries are unloaded in full cases from the side door on the body and empties are replaced on the pallet skids. On his return, the driver has the option of unloading immediately or leaving the unsold quantities, if any, in the truck overnight. The body is fully refrigerated and insulated sufficiently to allow this margin of protection. However, when the driver chooses to unload completely, he backs up to the dock and unloads 360 cases of empties, stacked on the eight pallets.

It is estimated that this system of operation is credited with reducing handling operations, risk of breakage, and man-power costs. Time in loading and unloading with this system (7 minutes to load and unload) has been stated to be about one-third of that in the old-type operations. *(12)* Another type of body which has become widely used during the last few years is a combination unit which carries two temperatures, one temperature for ice cream or other frozen foods, at well below zero in one section, and another temperature for milk or other dairy products, at approximately 35° to 40° in the other section. This is more commonly used in parts of the country where the population is widely scattered and where distances between towns are great. It is economical to use in these sections, because it not only has sufficient storage capacity to properly service the scattered routes but saves the expense of two drivers, which would be necessary if separate pieces of equipment were sent out, one handling only ice cream and the other only milk or dairy products.

We have built and are building many of these two-temperature bodies, with capacities of as high as 700 gallons of ice cream in the low-temperature section and 200 cases of milk in the high-temperature portion of the body. Some of these are refrigerated by heavy-duty hold-over plates, with separate compressors for the low- and high-temperature compartments. Others are equipped with a continuous refrigerating system which functions with the thin plates or, as we referred to them, $\frac{7}{8}$-inch or 1-inch plates, and the stand-by refrigerating unit, for use when the body is stored at night.

These are the developments and trends in refrigerated transportation equipment, as we see them, namely, *(1)* Larger-capacity bodies for wholesale delivery; *(2)* increased use of semi-trailers; *(3)* lighter-weight bodies; *(4)* lighter-weight refrigeration equipment; *(5)* continuous over-the-road refrigerating systems, with stand-by units for use at base of operations; *(6)* walk-in type ice cream and frozen products bodies; *(7)* pallet-loading in wholesale-milk bodies; *(8)* use of conveyors in loading semi-trailers, *(9)* combination two-temperature bodies.

Another unit has come into use during the last three or four years, called a portable slide-on refrigerated body, which may be installed without difficulty on a $\frac{3}{4}$-ton or 1-ton pick-up truck. It is adopted principally for house-to-house delivery in rural routes, and is available in two or three different sizes. It carries its own condensing unit and has proper hold-over facilities, to last during the time it is away from the plant.

Body manufacturers recognize the need of designing refrigerated bodies to meet the necessity of saving weight and cutting down loading and delivery time—all necessitated by changed conditions and the many different articles which comprise the payload.

TECHNICAL NOTES

INFLUENCE OF COTTAGE CHEESE STARTER ORGANISMS ON STRENGTH OF CURD AT TIME OF CUTTING

Hammer and Babel *(1)* reported a number of investigations on the proteolytic properties of butter cultures and noted that proteolysis was evident in freshly coagulated cultures. They suggested that proteolysis may benefit flavor production by the flavor organisms.

Recently, van der Zant and Nelson (2) studied the proteolysis of *Streptococcus lactis* and found definite increases in the free tyrosine and tryptophan content of milk after four hours of incubation.

While this property of the organism may be of little apparent significance in butter cultures, it may be related directly to certain aspects of the manufacture of cottage cheese.

In the study reported here, eight different commercial cultures were selected and carried under as nearly identical conditions as possible. All of these cultures when used as starters developed a whey acidity of 0.55-% in five and one-fourth to six hours and have been used satisfactorily in the manufacture of cottage cheese.

The effect of each culture on curd firmness was determined by measuring this property under conditions simulating the setting of milk during the manufacturing of cottage cheese. A Cherry-Burrell curd meter of the type recently made available was used for this purpose. Curd firmness under these conditions is believed to reflect the relative degree of proteolysis caused by different organisms of the cultures employed.

Four to five six-ounce cans were filled with 180 ml. of 11-% reconstituted nonfat dry milk. After adjusting to 90° F., 0.75-ml. of 1:100 commercial cottage cheese coagulator was added, with 7.5 ml. of the starter to be tested. The samples were held at 89° to 90° F. for about five hours and one sample was removed and tested for firmness and acidity of the whey. Depending upon the whey acidity, the remainder of the samples was removed at about 15-minute intervals and tested for curd strength and for acidity of the filtered whey. These results were then plotted and the curd strength interpolated for 0.55-% whey acidity.

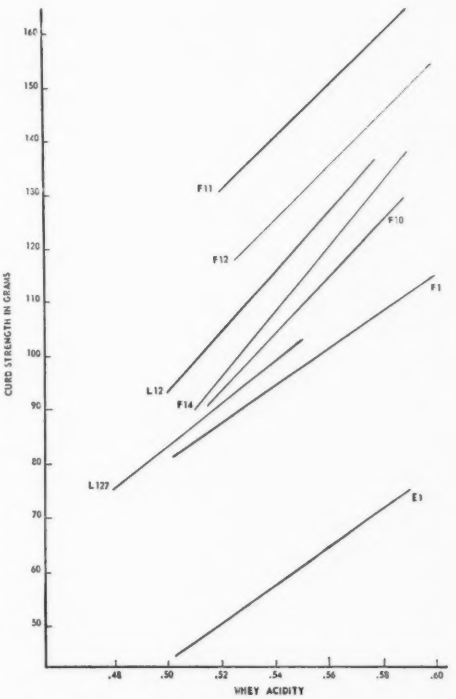

FIG. 1. Relation between whey acidity and rate of firming of cottage cheese curd for eight cultures.

TABLE 1

Curd strength of reconstituted nonfat dry milk (11%) when interpolated to 0.55-% whey acidity

Starter	Trial (Curd strength in gm.)				Hours to reach 0.55-% whey acidity
	1	2	3	Av.	
F 11	137	137	144	139	5.42
F 12	130	126	120	125	5.75
L 12	108	135	130	124	5.25
F 14	120	119	114	118	5.25
F 10	124	131	98	118	5.25
L 127	120	113	109	114	5.42
F 1	84	109	101	98	6.00
E 1	89	90	62	80	5.33

Table 1 shows the curd strength of the reconstituted nonfat dry milk at 0.55-% whey acidity. Each starter is fairly consistent in its ability to form curd of a given strength. There is no relationship between the firmness of the curd and time to reach 0.55-% whey acidity.

Figure 1 shows the relationship between whey acidity and curd firmness, produced by the different cultures during a constant time interval. For comparison, a curd strength of 50 gm. would be considered a soft coagulum by the cottage cheese maker. A curd strength of approximately 100 gm. would be considered normal, and a curd strength of 160 gm. would be considered firm. Starters developing acid at normal rates produced various curd strengths

in the milk at time of cutting. The various curd strengths appear to be reproducible for each starter. Figure 1 also shows that the rate of curd firming by the different starters varies linearly with the acid development over the range shown.

Results obtained in this study indicate that the starter used in the manufacture of cottage cheese significantly affects firmness of the curd at time of cutting. This presumably is due to variations in the proteolytic activity of different types of organisms present in the different cultures.

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PRELIMINARY FERTILITY RESULTS FROM THE PRESERVATION OF BOVINE SEMEN AT ROOM TEMPERATURES

The preservation of fertility of semen at room temperature would be of great economic importance and would serve to facilitate the increased use of artificial insemination, especially in areas where refrigeration is not available. Numerous attempts to store sperm at room temperature have not been successful, because of the limited sperm survival obtained *(1, 2, 3, 8, 9)*. Recent investigations at the University of Illinois, involving the effect of carbon dioxide on spermatozoa, have resulted in the development of a method which successfully preserves the motility and fertility of bovine semen for several days at room temperatures. Details concerning the effects of CO_2 on sperm motility, and the development of media for room-temperature storage, will soon be submitted for publication in this journal *(6, 7)*. The effects of CO_2 on sperm metabolism are being published elsewhere *(4, 5)*.

The preservation of bovine semen at room temperature has been achieved by incorporating CO_2 into a suitable diluent and hermetically sealing the diluted semen in ampules. The diluent was composed of 20.0 gm. sodium citrate dihydrate, 2.1 gm. sodium bicarbonate, 0.4 gm. potassium chloride, 3.0 gm. glucose, and 3.0 gm. sulfanilamide, dissolved (by heating to near boiling) in a liter of water distilled over glass. This solution was then cooled to room temperature and was saturated with carbon dioxide by bubbling the gas through it for ten minutes (or until the pH was reduced to about 6.35). After saturation with CO_2, 1,000 I.U. penicillin and 1,000 μg. dihydrostreptomycin sulfate per ml. of diluent were added, along with sufficient fresh egg yolk to make a final diluent consisting of ten per cent yolk. This diluent is called the Illini Variable Temperature (IVT) diluter.

The effectiveness of this new method for storing semen at room temperatures was tested by comparing nonreturn-to-service rates of cows bred with IVT-diluted semen versus yolk-citrate diluted semen, using a split-ejaculate technique. Split ejaculates of semen from two Holstein bulls of the Northern Illinois Breeding Co-op were diluted with the IVT diluent, so that each ml. contained approximately 25

TABLE 1

Comparative fertility of split ejaculates of two bulls when stored in the Illini Variable Temperature diluter (IVT) at room temperature and in 1:3 yolk-citrate diluter at 5° C.

	Semen in IVT diluter at room temperature			Semen in 1:3 yolk-citrate diluter at 5° C.	
Age of semen	Cows bred	28-39 day N.R.[a]	60-90 day N.R.	Cows bred	60-90 day N.R.
(days)	*(No.)*	*(%)*	*(%)*	*(No.)*	*(%)*
1-2	21	81.0	81.0	469	72.0
2-3	22	95.4	72.7	43	41.9
3-4	23	91.3	78.3	15	40.0
4-5	20	75.0	70.0	2	0
5-6	20	90.0	70.0	6	0
6-7	5	100.0	100.0	....	.....
Total	111	87.4	75.7	535	66.9
By bulls					
Ideal	55	85.5	70.9	338	63.1
Jule	56	89.3	80.4	197	73.6

[a] N.R. = nonreturn to service.

million sperm. One-ml. quantities of the diluted semen were sealed in glass ampules (2.2 ml. total capacity) and stored in the dark at room temperatures (65 to 80° F.). This semen was used to breed at least ten cows per day for the following five to six days. The procedure was repeated the following week, with a second technician making the inseminations with two additional semen samples from the same two bulls. The diluted semen taken to the field each day was kept in a polystyrene box to avoid sudden changes in temperature.

The major portion of each split ejaculate was diluted with the commonly used yolk-citrate diluent (one part egg yolk, three parts of a 2.9% solution of sodium citrate dihydrate; with three mg. sulfanilamide, 1,000 I.U. penicillin, and 1,000 μg. streptomycin/ml.), to contain not less than 25 million sperm per ml. This portion of each ejaculate was cooled and stored at 5° C. in accordance with standard practices and was used by all other technicians of the Co-op for routine inseminations.

Data from the preliminary field trial are summarized (Table 1). All of the cows bred with yolk-citrate diluted semen and all but six of the 111 cows bred with semen in the IVT diluter were first-service cows. The record of each cow bred with the IVT diluter was checked carefully at the farm, to insure the validity of the return-to-service dates. The data show that the fertility of the IVT-diluted semen did not decrease during six to seven days' storage at room temperature. In addition, the over-all nonreturn rate of 75.7% was nine per cent greater than the nonreturn rate for the same ejaculates in the routine yolk-citrate diluent stored at 5° C., even though the latter was only one to three days old when used. The improved fertility resulting from the use of the IVT diluter was evident with the ejaculates from both bulls. The number of cows returning to service after being bred with semen held at room temperature did not change more than expected between the 28- to 39-day check and the 60- to 90-day tabulation. Thus, there is no suggestion of a high embryonic death rate for that period.

The above data demonstrate that bovine semen can be stored at room temperature for several days without loss of fertility. Continued studies have shown that reasonable care in processing and handling are essential if the sperm are to withstand the rigors of this high temperature. A number of other factors affecting the survival of sperm at room temperature are being investigated.

ACKNOWLEDGMENTS

The authors are indebted to Dr. W. O. Nelson for his help with this manuscript and to the directors, management, and technicians of the Northern Illinois Breeding Co-op for their help with this investigation.

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PLASMA 17-HYDROXYCORTICOSTEROID LEVELS IN A DAIRY COW AFTER INTRAVENOUS ADMINISTRATION OF HYDROCORTISONE[1]

Considerable evidence suggests that the adreno-cortical steroids are secreted as free, unconjugated steroids. Conjugation of the steroids occurs mostly in the liver and results in urinary (or fecal) metabolites with different physical and chemical properties. Hechter *et al. (5)* used column and paper chromatographic methods to isolate both hydrocortisone and corticosterone from the blood of cattle.

Methods are available for the determination of both free *(11, 17)* and total *(2, 6, 14, 18)* 17-hydroxycorticosteroids (17-OH-CS) in human plasma, and Robertson and Mixner *(15)* have developed a method for the assay of free 17-OH-CS in bovine plasma.

The changes in plasma levels of 17-OH-CS after the administration of corticosteroids (or ACTH) have been used as a basis for judging the effects on steroid metabolism of epinephrine [Sandberg *et al. (16)*], ascorbic acid [Bacchus *(1)* and Haynes and Sheid *(4)*], the liver [Klein *et al. (7)*], and the thyroid [Levin and Daughaday *(8)*]. In none of these studies were developed adequate mathematical expressions for the rate of disappearance of exogenous (or endogenous) corticosteroids from the plasma.

Petersen and Wyngaarden *(13)* and Migeon *et al. (9)* injected 4-C^{14}-hydrocortisone into the blood of normal humans and followed the rate of disappearance of free steroid from the blood. A biological half-life of approximately 80 minutes was reported in each instance using first-order rate regression principles. Petersen and Wyngaarden *(13)*, in addition, used the chemical assay method of Silber and Porter *(17)* to follow the rate of disappearance of intravenously administered hydrocortisone free alcohol from the plasma of human subjects. The biological half-life using first-order rate regression principles averaged 115 minutes in 18 normal subjects, ranged from 160 to 800 minutes in eight cases having liver disorders of varying severity, and was of the order of 60 minutes in two thyroxtoxicosis patients.

This report presents the characteristics of the disappearance of hydrocortisone (free alcohol) from the plasma of a dairy cow after rapid intravenous infusion using first-order rate regression principles.

Materials and Methods

A six-months pregnant, lactating, mature Holstein cow weighing 666 kg. was used in this trial. Four hundred mg. of hydrocortisone (free alcohol)[2] in a solution of 5% ethanol was infused rapidly into a jugular vein. Blood samples were collected from the jugular veins at varying intervals of time during one hour after the infusion. The time at the beginning and end of the infusion was noted, as were all blood collections, in order to calculate the mean infusion and blood collection times.

Plasma free 17-OH-CS was determined by the method of Robertson and Mixner *(15)*, which method has an extraction efficiency of 81%.

Results and Discussion

Following the intravenous infusion of 400 mg. of hydrocortisone, the level of plasma 17-OH-CS fell rapidly during the first few minutes, after which the rate of fall was less rapid (Table 1 and Figure 1). The theoretical concentration of 17-OH-CS in the plasma at zero time, based on a plasma volume of 38.1 ml. per kg. of body weight, was 1,588.1 μg. %. The distribution of hydrocortisone from the plasma into body fluids and tissues, plus its actual disappearance due to conjugation, catabolism, and excretion, may account for the initially rapid disappearance of the steroid. It will be seen (Table 1) that an estimated 15.26% of the injected hydrocortisone free al-

TABLE 1

Disappearance of 400 mg. of 17-hydroxycorticosterone free alcohol from blood plasma of a cow

	Plasma 17-hydroxycorticosteroids			
Time after infusion	Actual assay values	Assay values adjusted for efficiency of extraction	Adjusted values minus preinjection values	Part of total dose present in plasma[1]
(min.)	*(μg.%)*	*(μg.%)*	*(μg.%)*	*%*
Preinjection	9.5	11.7	0	0
2.32	204.4	252.3	240.6	15.26
4.32	88.4	109.1	97.4	6.18
6.32	60.0	74.1	62.4	3.96
8.82	47.9	59.1	47.4	3.01
11.32	38.5	47.5	35.8	2.27
16.90	36.5	45.1	33.4	2.12
22.12	25.1	31.0	19.3	1.22
27.45	19.2	23.7	12.0	0.76
31.65	15.1	18.6	6.9	0.44
36.70	13.9	17.2	5.5	0.35
42.00	12.7	15.7	4.0	0.25
52.00	11.4	14.1	2.4	0.15

[1] Based on a plasma volume of 38.1 ml. per kg. of body wt., body wt. being 666 kg.

[1] Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers University, the State University of New Jersey, Department of Dairy Industry, New Brunswick. This work was supported in part by research grants from Swift and Company, Chicago, and Merck and Company, Rahway, New Jersey.

[2] Generously supplied by Laurent Michaud, Merck and Co., Rahway, New Jersey.

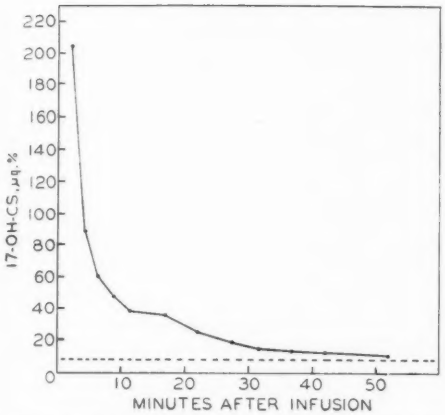

FIG. 1. Actual plasma concentrations of 17-OH-CS after the rapid intravenous administration of 400 mg. of hydrocortisone into a mature, lactating, pregnant Holstein cow. The dashed line represents the preinjection level of 17-OH-CS.

cohol remained in the plasma 2.32 minutes after the infusion. Migeon *et al. (9)* reported that from 15 to 20 minutes after the infusion of 4-C^{14} hydrocortisone, little radioactivity had been excreted into urine, bile, and feces, and that approximately 85% had moved from the blood stream into extracellular spaces or tissues. The slower rate of disappearance of the steroid which followed may be due to conjugation, catabolism, and excretion after distribution was complete.

Theoretically, the curve of disappearance of free 17-OH-CS from plasma follows the general equation, 17-OH-CS, $\mu g. \% = a_1 e^{-bt} + a_2 e^{-b't}$, where $-b$ = slope (rate constant) of the curve of distribution of hydrocortisone into its volume of distribution, and $-b'$ = slope (rate constant) of the curve of disposal of hydrocortisone [see O'Neal *(12)* for similar studies with *dl*-thyroxine and plasma protein-bound iodine]. In this instance, the rate of distribution of the steroid into the tissues and extracellular spaces was so rapid that the consideration of the mixing time of the steroid in the blood prevented obtaining adequate data upon which to base an analysis of the distribution curve. A rough estimate of the biological half-time for the mixing of the hydrocortisone into its volume of distribution was 0.7-minute.

The curve of disposal of hydrocortisone (Figure 2), presumably representing the conjugation, catabolism, and excretion of the steroid, has a slope of 0.077 and a biological half-life of nine minutes. The slope of the disposal curve was calculated by the method of least squares, in which the time in minutes after the infusion was the X-variable and the natural logarithm of the 17-OH-CS value (corrected for extraction efficiency) minus the preinjection value (corrected for extraction efficiency) was the Y-variable (Table 1). The coefficient of correlation between the pair of variables was -0.99.

The volume of distribution of hydrocortisone may be calculated from the following equation: V. D., ml. = amount of hydrocortisone infused, $\mu g. / a_2 \div 100$ in which a_2 = concentration of 17-OH-CS (102.85 $\mu g. \%$) of the disposal regression curve (Figure 2) at zero-time. The volume of distribution was calculated to be 388,916 ml., or 58.4% of body weight. The calculation of a volume of distribution of the steroid would seem to be valid only if the steroid was rapidly infused, as was done in this study, giving an accurately timed "zero"-point.

The biological half-life of hydrocortisone in the cow (nine minutes) obtained in this study was much shorter than the half-life of 115 minutes in the human subjects, reported by Petersen and Wyngaarden *(13)*. This may be related to a possible difference in the primary mode of excretion of certain steroids in the two species, which is largely via the urine

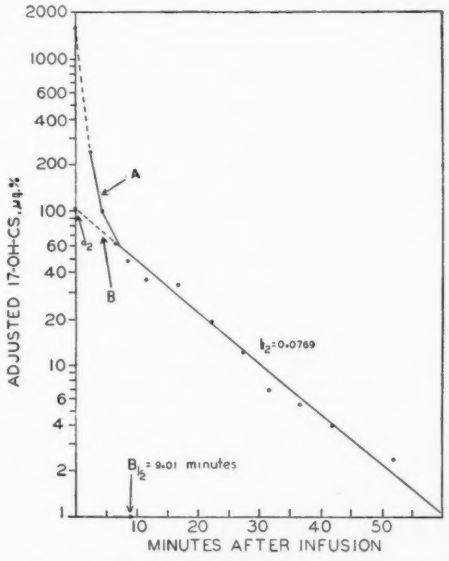

FIG. 2. Analysis of the curve of disappearance of 17-OH-CS from blood plasma after the rapid intravenous injection of 400 mg. of hydrocortisone free alcohol. Curve A is a semilogarithmic plot of the data in Figure 1, adjusted for the efficiency of extraction and minus the preinjection level of 17-OH-CS in the plasma (Table 1). The dashed portion of this curve is an extension to the theoretical level of 17-OH-CS at zero time. Curve B represents the rate of disappearance of free 17-OH-CS from plasma, due to catabolism and excretion, with a slope of 0.0769 and a biological half-life of 9.01 minutes.

in the human [Petersen and Wyngaarden *(13)*] and is possibly via the feces in cattle [Miller *et al. (10)* and Casady *et al. (3)*].

Results of Petersen and Wyngaarden's *(13)* work with humans, and the present report with cattle, indicate that the biological half-life and the slope (rate constant) of the disappearance of free hydrocortisone from plasma would be useful measures of steroid metabolism.

Summary

First-order rate regression principles were used to characterize the rate of disappearance of hydrocortisone free alcohol from the blood plasma of a dairy cow. Four hundred mg. of hydrocortisone were infused rapidly and intravenously into a mature, lactating, Holstein cow during the sixth month of pregnancy. The disappearance of the free steroid from the blood plasma had two phases, the first a rapid disappearance with an extremely short half-life (estimated at about 0.7-minute) and the second, slower disappearance with a slope (rate constant) of 0.0769 and a biological half-life of 9.01 minutes. Presumably, the first phase represented the rapid distribution of the steroid into the body fluids and tissues, and the second phase represented the metabolism and excretion of the steroid. A volume of distribution of 58.4% of body weight was calculated for this steroid.

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ON MAINTENANCE OF THE CORPORA LUTEA OF THE BOVINE WITH LACTOGEN

A number of investigators have reported a luteotrophic function of lactogen in the rat. (For review of earlier, related work consult Reference 4.) Moore and Nalbandov *(4)* present evidence to indicate that lactogen has luteotrophic activity in the ewe. However, Bradbury *et al. (2)* and Hisaw *(3)* failed to maintain corpora lutea of women or monkeys with lactogen. This study was undertaken to ascertain the effects of lactogen in maintenance of corpora lutea of intact cattle.

Animals used in this experiment were Guernsey or Holstein heifers, 15 to 22 months of age. Usually, each heifer was observed for several estrous periods, to determine the regularity of the cycle before treatment with lactogen. The lactogen preparations used in four of the trials were prepared by one of us (W. H. Mc.), according to the method of Bates *(1)*. The amount of hormone in these preparations is expressed as gm. equivalent. This represents the gm. of original acetone desiccated dry powder of whole pituitary (sheep) from which the hormone was extracted. A commercial lactogen preparation was used for five trials.[1] Daily subcutaneous injections of the preparations were made beginning three to 17 elapsed days after the beginning of estrus. The dose of hormone injected was four to eight gm. eq. and 300 to 1,800 I. U. Rectal examinations were made at four-day intervals, to determine the size and status of ovarian structures.

[1] The commercial lactogen was supplied by Irby Bunding, Armour Laboratories, Chicago.

Results are presented (Table 1). All heifers returned to estrus within a range of 19 to 26 days while being injected with lactogen, and no prolongation from treatment was indicated on those for which the previous estrous cycle length was known. Rectal palpations indicated that the corpora lutea regressed and follicles developed as would be expected. (In one heifer, 211-G, the follicle ruptured while being palpated early in estrus). Lactogen does not appear to have a luteotrophic action in the intact bovine, with the dosages administered beginning at different times in the estrous cycle.

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TABLE 1

Effect of lactogen on maintenance of the corpus luteum

Animal No.	Lactogenic hormone preparation	Amount of hormone injected daily	Day of cycle injections began	Length of "treatment" estrous cycle	Length previous estrous cycle
553H	122	4 gm. eq.	9	26	—
552H	50-53	8 gm. eq.	9	19	—
	50-53				
552H	122	8 gm. eq.	9	21	19
564H	133	300 I. U.	9	22	21
195G	759-014-A	600 I. U.	3	24	22
199G	759-014-A	600 I. U.	4	20	20
678H	P10011	900 I. U.	3	20	21
211G	P10011	1,800 I. U.	17	22[a]	22
49H	P10011	1,800 I. U.	17	23	22

[a] An 18-mm. follicle, with papilla, ruptured with moderate pressure early in estrus.

PRELIMINARY OBSERVATIONS ON THE EFFECT OF SLIGHT PROTEOLYSIS OF FLUID MILK ON WETTABILITY OF THE RESULTING DRIED MILK[1]

In 1898 Bachaus *(2)* described a procedure for the production of soft curd milk, which involved treatment of the milk with proteolytic enzymes. Conquest *et al. (3)* and others *(7, 10)* have also carried out further work in this connection.

Tarassuk and Nury *(11)* subjected evaporated milk to a limited enzymatic proteolysis and observed a marked increase in the viscosity of the milk after subsequent sterilization. The increase in the viscosity was found to be directly related to the lowering of the stability of the milk towards heat.

Several workers have shown that mild treatment of milk with proteolytic enzymes before pasteurization gives some protection against the development of oxidized flavors *(1, 4, 5)*.

This paper reports the results of preliminary experiments on the effects of treating fluid milk with proteolytic enzymes, prior to drying, on the wettability of whole milk powder.

The fat content of freshly drawn milk (3.8% milkfat) was adjusted to 3.3% with skimmilk. Three 500-ml. portions of this milk were treated with Rhozyme P11 (Rhom and Haas) under the following conditions: The temperatures of samples 1, 2, and 3 were adjusted to 60° C., 65° C., and 70° C., respectively, before the addition of the enzyme (0.17 g; 1% of milk protein). The temperatures were then increased at the rate of one degree per minute until a temperature of 75° C. was reached. This temperature was maintained for 15 minutes. A fourth sample, which served as a control, was heated to 60° C., and the temperature was raised to 75° C. at the rate of one degree per minute and was maintained at this temperature for 15 minutes before cooling.

The viscosity of the four samples did not differ appreciably. A taste panel *(9)* showed a slight preference for the milk that had been treated at 60° C. (Sample 1). This preference indicated that Sample 1 had a more creamlike flavor. The tyrosine values *(6, 10)* expressed as milligrams tyrosine per liter were as follows: control, 24; Sample 1, 54; Sample 2, 46; Sample 3, 32.

One hundred pounds of milk was divided into two equal portions. One was heated to 60° C., Rhozyme P11 (7.94 g.) was added to one 50-lb. portion (treated milk) and no enzyme was added to the other portion. The milk was heated by the same procedure as that followed with Sample 1 of the previous experiment. After the heating period, the milk was cooled to 60° C. and was homogenized at 2,300 lb. pressure, before condensing it in a vacuum pan to approximately 36% solids.

The milk was dried in a laboratory-type, Niro Spray Drier operated under the following conditions: air pressure on the atomizer 6.0-6.5 kg/cm^2; inlet air temperature, 190°-200° C.; outlet temperature, 85°-95° C. Table 1 shows the moisture content *(8)*, the solubility index (ADMI), the tyrosine value, and the taste rating of the two samples.

The following procedure was used to check the wettability of the two samples: 10 g. of milk powder was placed on the surface of water at 25° C. in a 250-ml. cylinder. Photographs were taken immediately after the addition of the powder (Figure 1a), 30 minutes after the addition (Figure 1b), and 1 hour after the addition (Figure 1c). The photographs show that the treated sample was appreciably more wettable than the untreated sample.

The results of this experiment were confirmed in detail by complete repetition of the work on two other occasions.

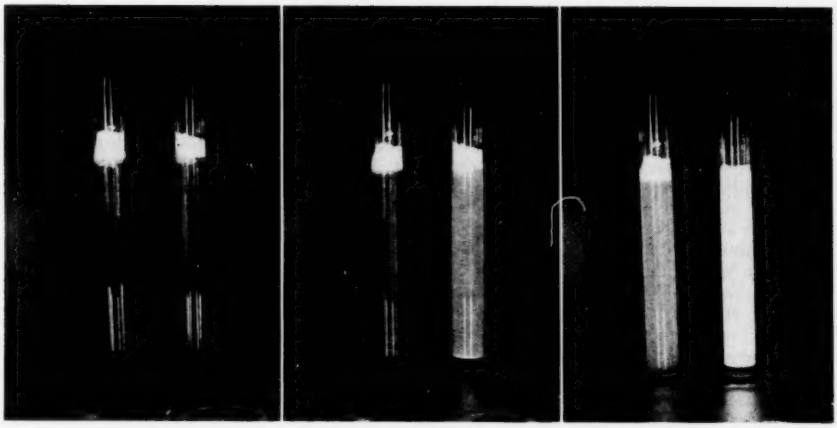
The results of this investigation show that the wettability of milk powder may be improved by treatment of the milk, prior to drying, with proteolytic enzymes. The mild enzyme treatment would affect primarily the surface of the protein molecule *(10)*. Such changes in the surface of the molecule would be likely to alter its hydrophilic properties, and

[1] Contribution from the Faculty of Agriculture, McGill University, Macdonald College, Que., Canada. Journal Series No. 396.

TABLE 1

The effects of enzyme treatment on the properties of whole milk powder

Dried milk	Moisture content	Solubility index (ADMI)	Tyrosine value (Mg. Tyrosine/liter)	Taste criticism
Treated	2.1	0.4 ml.	55	1. Less chalky tasting and more creamy than untreated sample
Untreated	2.0	0.3 ml.	23	2. Taste indistinguishable from that of freshly prepared, commercial whole milk powder



(a) Immediately (b) 30 minutes (c) One hour

FIG. 1. The effect of enzymic treatment on the wettability of whole milk powder. The cylinder on the left in each photograph contains the untreated sample, and the one on the right, the treated sample.

the present observations suggest that, of these properties, the wettability is appreciable and advantageously modified.

ACKNOWLEDGMENTS

The authors wish to thank the Quebec Research Council and the Department of National Defense for research grants which have defrayed part of the expense of this investigation. One of the authors (J.P.J.) wishes to thank the Granby Co-op., P. Q., for a fellowship.

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A SPECTROGRAPHIC ANALYSIS OF TWO PROTEIN FRACTIONS OF THE FAT-GLOBULE MEMBRANE OF NORMAL COW'S MILK

The most closely associated protein materials of the fat-globule membrane of normal cow's milk have been separated into two fractions, based upon their solubility in a 0.02-*M* sodium chloride solution, subsequent to an ethanol–ethyl ether treatment *(3)*. The ash content of the soluble and insoluble fractions, following exhaustive dialysis against running tap-water and four changes of distilled water over a period of 72 to 96 hours, was 7.06 and 2.08, respectively. These values are considerably higher than corresponding values for other milk proteins. Distribution of minerals between the two protein fractions becomes of academic interest, in view of our observations associating the alkaline phosphomonoesterases with the soluble protein fraction, and xanthine oxidase with the insoluble fraction *(3)*. The ash from these proteins presented an excellent opportunity for a spectrographic analysis of their constituent elements.

TABLE 1

Spectrographic analysis of the ash of the fat-globule membrane proteins

Element[a]	Soluble[b] fraction	Insoluble[b] fraction	Total[c] membrane-protein material
Aluminum	Trace	Trace	Trace
Calcium *(%)*	0.603	0.319	0.444
Copper *(p.p.m.)*	132	82	104
Iron *(p.p.m.)*	91	510	326
Magnesium *(%)*	0.548	0.227	0.368
Manganese *(p.p.m.)*	11	11	11
Molybdenum *(p.p.m.)*	5	125	72
Phosphorus *(%)*	0.416	0.289	0.344
Silver	Trace	Trace	Trace
Zinc *(p.p.m.)*	17	22	20

[a] Concentration estimated on basis of anhydrous protein.

[b] Based upon solubility in 0.02-*M* NaCl solution.

[c] Calculated on distribution of 44% soluble and 56% insoluble.

The residual ash of the protein samples was dissolved in four milliliters of 1:1 HCl. One milliliter of cobalt chloride solution was added to the solution as an internal standard. Aliquots of these solutions were dried on carbon electrodes and the residues excited in an a.c. spark with a Hilger large-prism spectrograph for the elements reported, with the exception of zinc, which was excited in a d.c. arc. The spectrographic plates were evaluated with a Jarrell-Ash microphotometer, using fruit tree-leaf standards for all elements except molybdenum, for which a standard was prepared especially.

Qualitative and approximate quantitative data for the elements detected are presented (Table 1). Where comparisons are possible, the fat-globule membrane–protein fractions contain mineral elements in higher concentration than reported for whole milk *(1, 2)*. These observations suggest that many of the trace elements reported in whole milk are concentrated on the fat-globule membrane, possibly as constituent elements of various enzymes or metabolic complexes.

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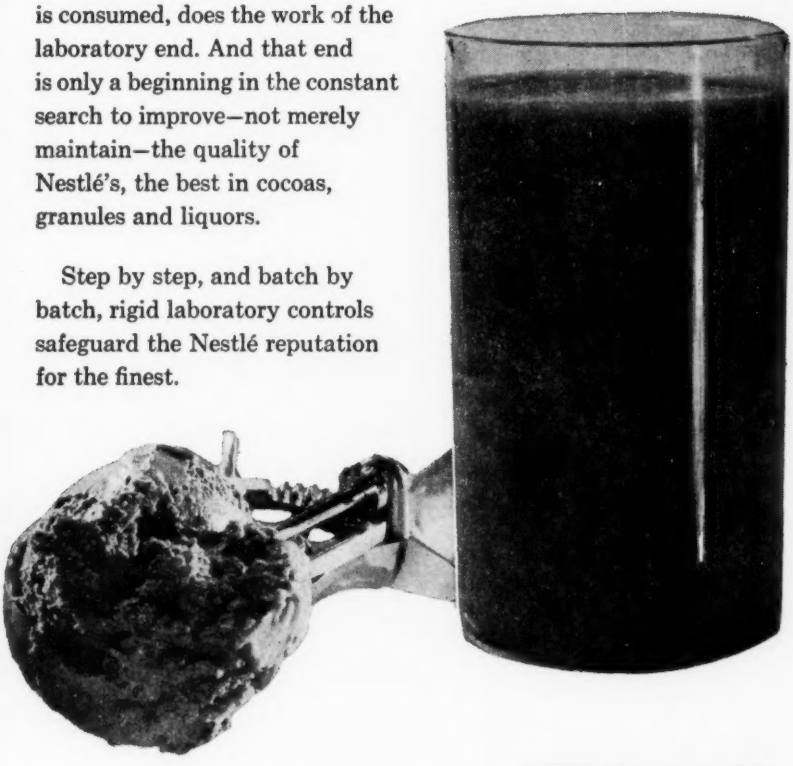

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